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Synthesis of Diaminopimelic Acid (DAP) and Analogues; Mechanistic Studies on DAP Aminotransferase, Epimerase and Dehydrogenase

by

Yong Gao ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Synthesis of Diaminopimelic Acid (DAP) and Analogues; Mechanistic Studies on DAP Aminotransferase, Epimerase and Dehydrogenase submitted by Yong Gao in partial fullfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Three synthetic approaches to biologically important *meso*-diaminopimelic acid (DAP) (**1a**) and its *N*-selectively protected derivative (2*S*, 6*R*)-2-(*N*-Cbz-amino)-6-(*N*-Boc-amino)heptane-1,7-dioic acid (**42**) were investigated. Although the first route *via* olefin metathesis of 3-((2*R*)-2-(*N*-Boc-amino)-4-pentenoyl)propyl *N*-Cbz-L-vinylglycinate (**19a**) was blocked by rapid isomerization of vinylglycine moieties **19a** to the α,β-unsaturated ester **19**, an analogous model study of 2-((2*R*)-2-(*N*-Boc-amino)-4-pentenoyl)ethyl *N*-Cbz-L-vinylglycinate (**6**) showed that this route can potentially be used to generate 2,7-diaminosuberic acid and other selectively protected bis-amino acids. An alternative approach *via* an asymmetric ene condensation of methyl (*S*)-4-(phenylthio)allylglycinate (**30**) and methyl glyoxylate catalyzed by a chiral bis(oxazoline)-copper complex **31** gave dimethyl (2*S*, 6*S*)-6-(*N*-Cbz-amino)-2-hydroxy-4-(phenylthio)-4-heptene-1,7-dioate (**33**) in a 94:6 isomeric ratio. Nickel boride removal of sulfur and the double bond in the presence of Cbz group generated (2*S*, 6*S*)-6-(*N*-Cbz-amino)-2-hydroxyheptane-1,7-dioate ((2*S*)-**23**). The third method, involving asymmetric reduction of dimethyl (6*S*)-2-keto-6-(*N*-Cbz-amino)pimelate (**50**) with (*S*)-binaphthol-ruthenium catalyst as the key step gave (2*S*)-**23** with 79:21 isomeric ratio. The Mitsunobu substitution of (2*S*)-**23** with *N*-*tert*-butyl ((2-(trimethylsilyl)ethyl)sulfonyl) carbamate (**32**) followed by several deprotection steps gave the desired **42** and **1a**.

DAP aminotransferase (DAP AT) was incubated with a number of 4-, 5-, 6- and 7-carbon amino acids as well as two DAP hydrazino analogues. The preliminary biological tests of these compounds against DAP AT show: 1) the active site for the amino transfer reaction has strong stereochemical, and structural group preferences for substrates or inhibitors; 2) the nitrogen atom at the reactive side of DAP compounds is necessary for recognition; 3) 2-*N*-Cbz-ornithine (**73**) is an alternative substrate; 4) the enzyme may have more than one binding site.

Several isoxazoline compounds were synthesized and tested as transition state analogues toward DAP epimerase (DAP EP) and DAP dehydrogenase (DAP DH). Although none of these compounds demonstrated potent inhibitory activity against DAP EP, the regio- and stereochemistry of isoxazoline rings as well as the remote amino acids of these analogues play important roles in interaction with DAP DH. X-ray crystallographic studies of DAP DH incubated with one of these isoxazolines, (2*S*, 5'*S*)-2-amino-3-(3-carboxy-2-isoxazolin-5-yl)propanoic acid (**99**) show that **99** binds to the enzyme in a competitive fashion but with an unexpected orientation.

To my wife and parents

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LIST OF ABBREVIATIONS

[α]	specific rotation
Anal.	analysis
APT	attached proton test
aq	aqueous
AT	aminotransferase
atm	atmosphere
AZIDAP	2-(4-amino-4-carboxybutyl)-2-aziridine-carboxylic acid
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
Bop-Cl	*N,N*-bis-(2-oxo-3-oxazolidinyl)phosphinic chloride
bp	boiling point
br	broad
tert-Bu	*tert*-butyl
n-BuLi	*n*-butyl lithium
calcd	calculated
Cbz	benzyloxycarbonyl
Cbz-Cl	benzyl chloroformate
CI	chemical ionization

concd	concentrated
COSY	correlated spectroscopy
δ	chemical shift in parts per million downfield from TMS
d	doublet
DAP	2,6-diaminopimelic acid
DCC	1,3-dicyclohexylcarbodiimide
d.e.	diastereomeric excess
DEAD	diethyl azodicarboxylate
DEAE	diethylaminoethyl
DEAEA	2-diethylaminoethylamine
DH	dehydrogenase
DIBAL	diisobutylaluminum hydride
DMAP	4-*N,N*-dimethylaminopyridine
DMAP.HCl	4-*N,N*-dimethylaminopyridine hydrochloride
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
e.e.	enantiomeric excess
EI	electron impact ionization
ESI	electron spray ionization
EP	epimerase

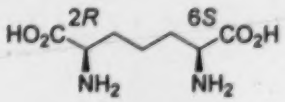
FAB	fast atom bombardment
GlcNAc	*N*-acetylglucosamine
HPLC	high performance liquid chromatography
IC_{50}	concentration at 50% inhibition
IR	infrared
J	coupling constant
k_{cat}	turnover number
K_i	inhibition constant
K_m	Michaelis-Menten constant
LDA	lithium diisopropyl amide
LHMDS	lithium hexamethyldisilazane
m	multiplet
m/z	mass to charge ratio
MCPBA	*m*-chloroperbenzoic acid
MHz	megahertz
mp	melting point
MS	mass spectrometry
MurNAc	*N*-acetylmuramic acid
MW	molecular weight
$NADP^+$	nicotinamide adenine dinuclotide phosphate, oxidized form
NADPH	nicotinamide adenine dinuclotide phosphate, reduced form
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect

Nu	nucleophile
PLE	pig liver esterase
PLP	pyridoxal phosphate
ppm	parts per million
Py	pyridine
Py-Bop	(benzotriazole-1-yl-oxy)-trispyrrolidino-phosphonium hexafluorophosphate
q	quartet
qui	quintet
R_f	retention factor
RP	reverse phase
rt	room temperature
s	singlet
t	triplet
TBAF	tetrabutylammonium fluoride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THDP	tetrahydrodipicolinate
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N,N,N',N'*-tetramethylethylenediamine
TMS	tetramethylsilane
TMSI	trimethylsilyl iodide

Tris-HCl	tris-(hydroxymethyl)aminomethane hydrochloride
p-TsOH	*p*-toluenesulfonic acid
U	unit
UV	ultraviolet
V	velocity
V_{max}	maximal enzyme velocity

Chapter 1 Importance of DAP and DAP Enzymes

Diaminopimelic acid (DAP) occurs as a constituent of bacterial toxins, immunoadjuvants, sleep inducing factors, and antitumor agents.[1-7] More importantly, DAP and its biosynthetic product L-lysine[8,9] are key compounds used in the construction



meso-DAP (**1a**)

of bacterial cell wall peptidoglycan for Gram-negative and Gram-positive bacteria,[10,11] respectively. The macromolecular peptidoglycan is responsible for maintaining the strength and rigidity of the cell wall and for protecting the cell from lysis under ordinary hypotonic conditions in its environment.[12,13]

The peptidoglycan consists of linear glycan strands of alternating β-1,4 linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc)[12,13] (Figure 1).

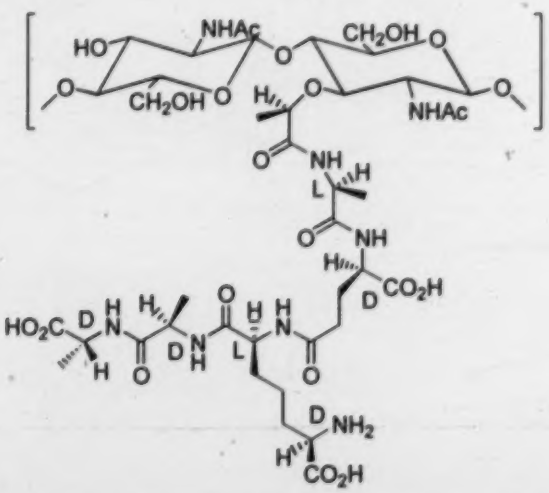


Figure 1. Peptidoglycan Monomer Unit in Gram-negative Bacteria

MurNAc residues are substituted on the D-lactyl group by pentapeptide units generally having the composition L-alanine, γ-D-glutamate, a diamino acid such as *meso*-DAP (**1a**) or L-lysine, followed by two D-alanines. To give peptidoglycan its necessary insolubility and rigidity, the glycan strands are crosslinked by peptide bridges. These bridges may involve the direct formation of a peptide bond between the penultimate D-alanine of one peptide with the free ω-amino group of the diamino acid (L-lysine or *meso*-DAP) in an adjacent glycan chain, or an additional intervening amino acid residue or peptide (Figure 2). As well as its involvement in cross-linking adjacent peptide chains, DAP is also utilized to link lipoprotein by amide bond formation between the L-carboxyl group of DAP and the ω-amino group of lysine residue on the lipoprotein which is known to be attached at about every 10th peptidoglycan chain.[14]

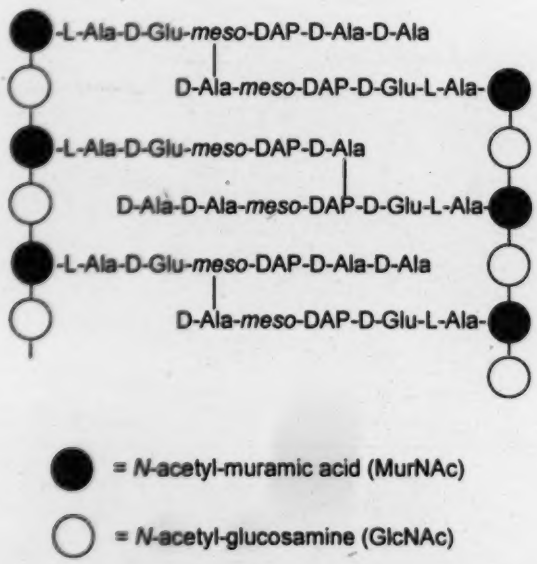


Figure 2. Crosslinking of Peptidoglycan in *E. coli.*

Due to the key roles of peptidoglycan to bacteria, a large number of existing antibiotics function by inhibiting its biosynthesis (Table 1).[15,16] Many target the metabolism of D-amino acids since the human body does not utilize them. For example,

Target	Antibiotic
Alanine racemase	alaphosphin, D-cycloserine
D-Ala-D-Ala ligase	D-cycloserine
Binding of peptidyl-D-Ala-D-Ala	Vancomycin
GlcNAc enolpyruvyl transferase	Phosphonomycin
Translocation across membrane	Bacitracin, tunicamycin
Transpeptidation of peptidoglycan	β-lactams

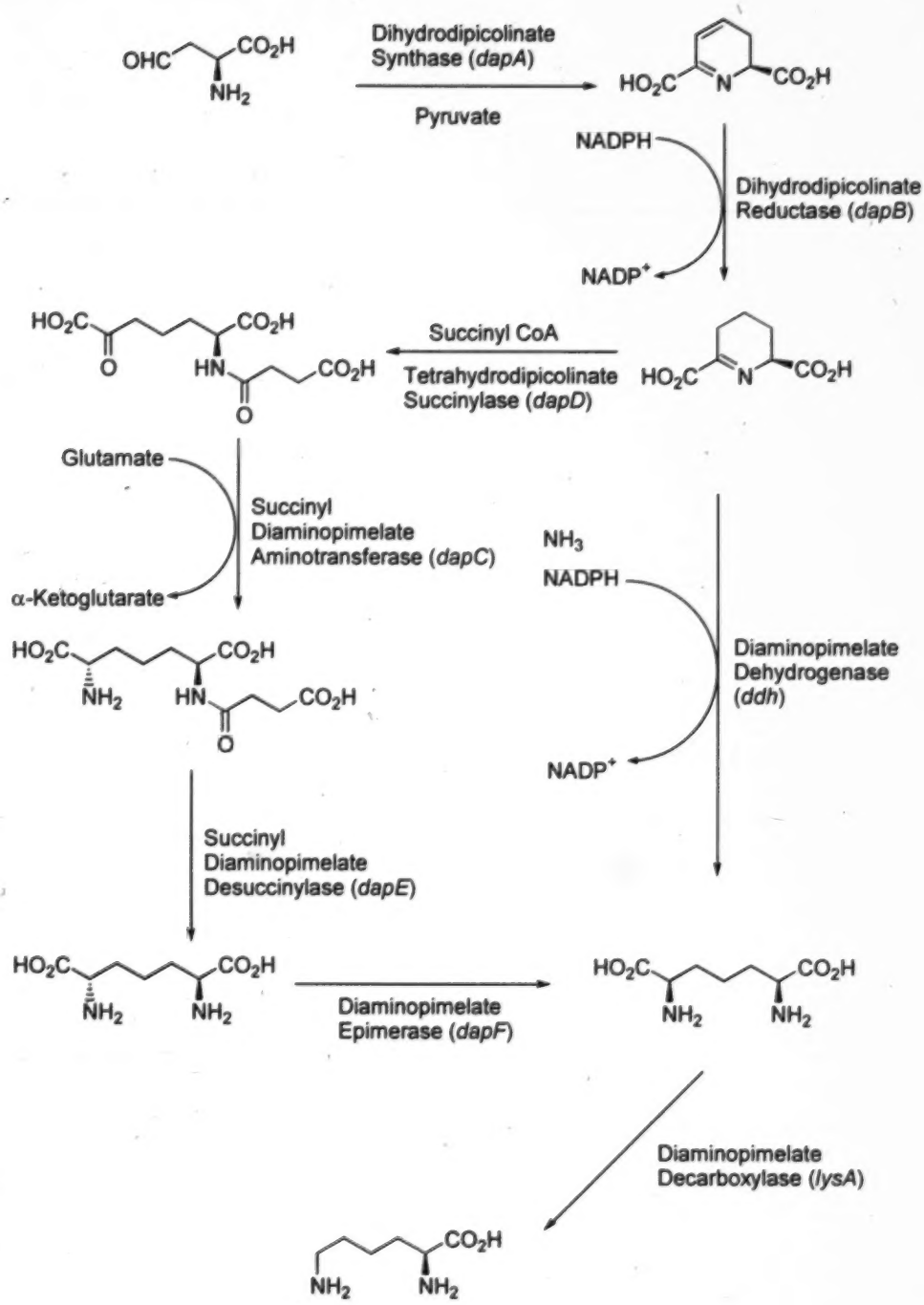
Table 1. Peptidoglycan Biosynthesis as a Target for Antibiotics

alaphosphin and D-cycloserine inhibit the biosynthesis of D-alanine, and vancomycin binds to peptidyl-D-Ala-D-Ala. There are relatively few antibiotics with structures related to that of DAP, and of these none have any therapeutic utility.[17] However, in the past several years, the rapid emergence of bacterial resistance to conventional antibiotics[11,18] has stimulated the interest in developing alternative antimicrobial agents, especially of those inhibiting DAP-metabolizing enzymes.[19-25] Such DAP-related antibiotics may have low mammalian toxicities since mammals lack the DAP biosynthetic pathway and require L-lysine in their diet.

There are three slightly different approaches for the biosynthesis of DAP and L-lysine by bacteria.[9,26] Two pathways involve DAP succinylase and DAP dehydrogenase, and share aspartate semialdehyde, L-dihydrodipicolinate and L-tetrahydrodipicolinate (L-THDP) as common intermediates (Scheme 1). The third route uses *N*-acetylated instead of *N*-succinylated intermediates, proceeds through an identical series of steps, and is extremely limited in its bacterial distribution. The deacylation of *N*-acetyl-DAP has only been described in *Bacillus* species, with the acetylase pathway of *B. megaterium* being the most well characterized system.[27,28] The pathway utilizing DAP dehydrogenase (*ddh*) to directly convert L-THDP to *meso*-DAP by a NADPH and ammonia-dependent reductive amination reaction has only been discovered in several bacteria like *Corynebacterium glutamicum, Bacillus sphaericus* and a *Pseudomonas* and *Brevibacterium* species.[26-29]

However, most bacteria use the "succinylation pathway",[9,29,30] which involves *N*-succinylation of the nitrogen of L-tetrahydrodipicolinate by succinyl-CoA. The resulting *N*-succinyl α-keto acid acquires a second amino acid center of L-configuration in a process catalyzed by DAP aminotransferase (*dapC*). This is followed by a de-succinylation reaction to give LL-DAP. The epimerization of LL-DAP is catalyzed by the *dapF*-encoded DAP epimerase to give *meso*-DAP, which undergoes a decarboxylation reaction to generate L-lysine.

Among DAP-metabolizing enzymes, particularly interesting are DAP aminotransferase (*dapC*), DAP epimerase (*dapF*) and DAP dehydrogenase (*ddh*), all of



Scheme 1. Lysine Biosynthesis

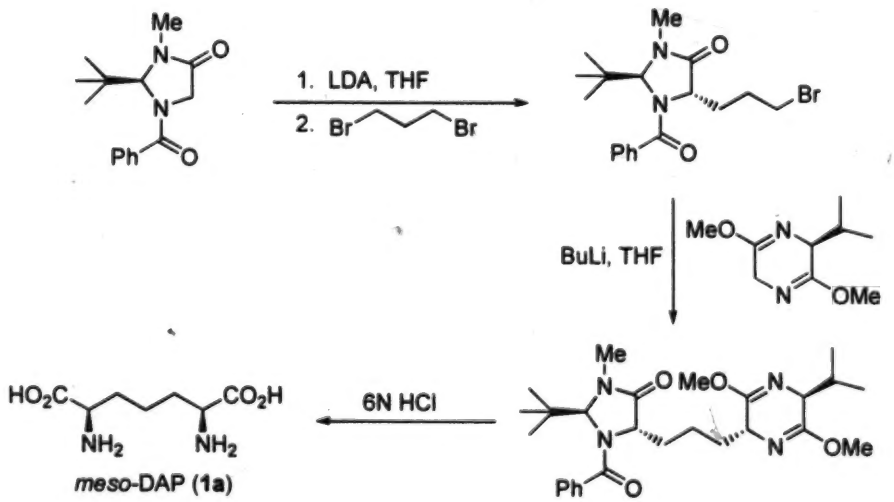
which have some unusual characteristics. DAP aminotransferase occurs in DAP biosynthesis as well as in L-arginine biosynthesis; DAP epimerase is one of the rare examples of non-PLP-dependent amino acid epimerases; and DAP dehydrogenase is the only dehydrogenase enzyme known to generate a D-stereocenter.

The goals of this project are: (1) to develop several synthetic approaches to selectively *N*-protected DAP derivatives that allow facile generation of analogues for enzyme inhibition studies; (2) to explore the mechanisms of DAP aminotransferase, epimerase and dehydrogenase. This project will assist the future work in this area and help us to understand a large number of homologous enzymes such as alanine aminotransferase, and glutamate racemase for the metabolism studies on other amino acids.

Chapter 2 Stereospecific Synthesis of *meso*-DAP and Its *N*-Selectively Protected Analogues

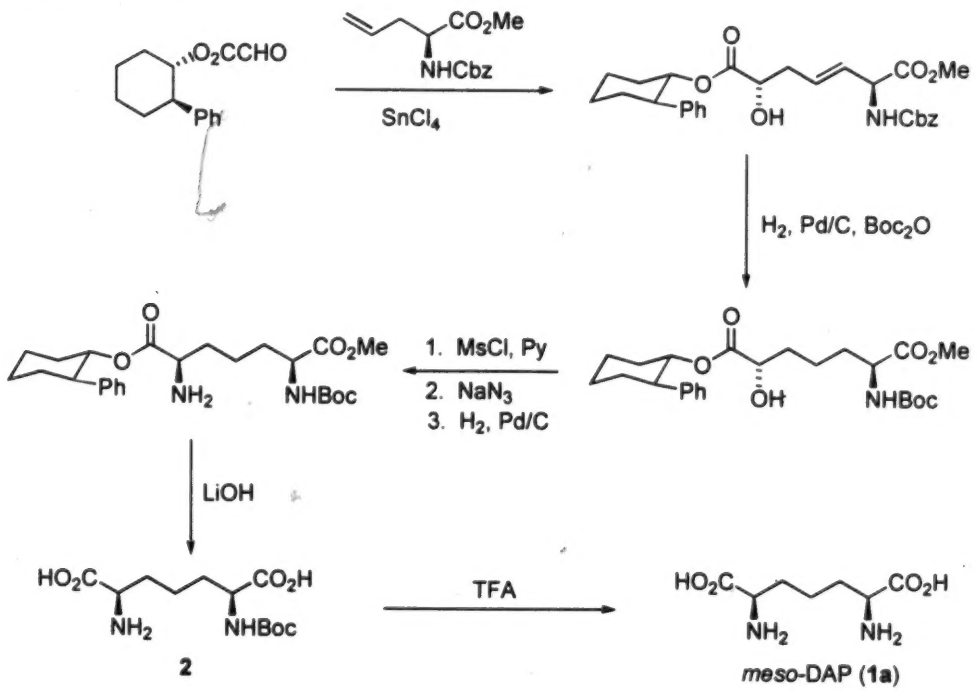
In addition to studies on the function and genetic organization of DAP-related enzymes,[31-38] considerable effort has focused on the synthesis of the DAP isomers and their analogues.[39-50] However, there is still a need to develop synthetic methodologies that provide DAP and its derivatives stereospecifically with complete orthogonal protection on both the amino and carboxylate groups. Manipulation of these protecting groups permits modification to generate inhibitors of DAP enzymes and assists synthesis of peptides that mimic the peptidoglycan structures.

To achieve this goal, several methods have been examined in our laboratory for the synthesis of *meso*-DAP and its *N*-protected derivatives, including both alkylation of chiral reagents (Scheme 2)[51] and the use of a chiral auxiliary to provide asymmetric



Scheme 2.

induction in an ene reaction (Scheme 3).[52,53] The dialkylation approach gives *meso*-DAP with 90% d.e., but fails to generate the selectively *N*-protected analogues. The alternative synthesis of DAP derivatives using chiral auxiliaries in Scheme 3 produces *meso*-DAP



Scheme 3.

with 72% d.e. and affords a desired intermediate **2**. However, this method is not satisfactory due to the relatively low asymmetric induction and additional steps required for introduction and removal of the chiral auxiliary. In this chapter, we describe three different approaches to *meso*-DAP and its analogues with selective protection on amino functions using optically pure L-allylglycine as the starting material.[53] One route, potentially applicable to a variety of unusual molecules with amino acid moiety at either

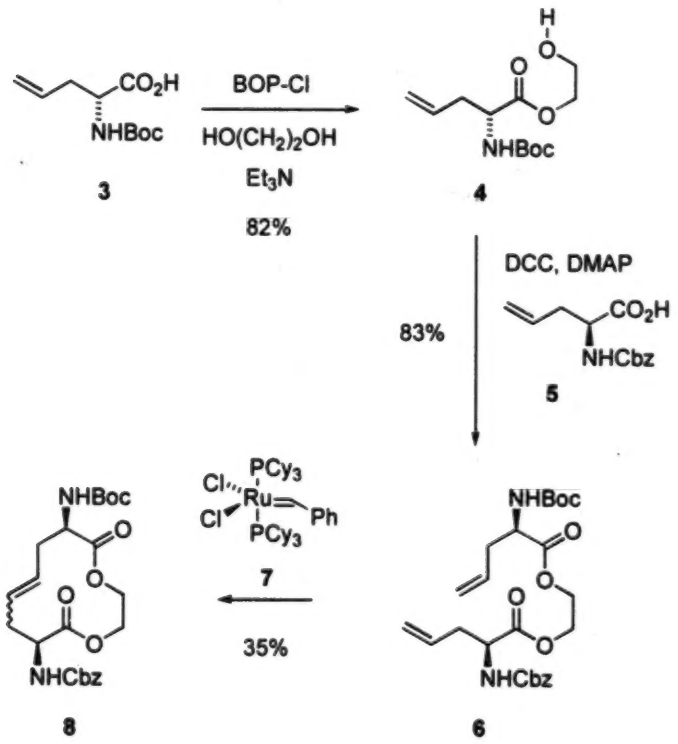
end, employs a ring-closing olefin metathesis reaction. The second approach utilizes an asymmetric ene condensation catalyzed by a chiral Lewis acid. Finally, the third approach adopts an asymmetric hydrogenation of an α-keto ester.

Olefin Metathesis Approach to DAP and 2,7-Diaminosuberic Acid

The successful application of olefin metathesis reactions for the synthesis of cyclic peptide derivatives by Grubbs and coworkers[54-56] suggests this methodology as a possible avenue to selectively-protected DAP. Since 12-membered rings can often be formed quite readily, it became apparent that linking two differentially *N*-protected amino acids bearing terminal olefins as esters of the appropriate chosen diol would permit metathesis cyclization with desired concomitant carbon-carbon bond formation. To test this, *N*-Boc-D-allylglycine (**3**) and *N*-Cbz-L-allylglycine (**5**) were sequentially esterified onto ethylene glycol to form diester **6** (Scheme 4). Reaction with the Grubbs catalyst **7** produces the cyclic olefin **8**, a protected derivative of 2,7-diaminosuberic acid in modest (35%, not optimized) yield.[53] It was assumed that ester hydrolysis and hydrogenation under standard conditions would generate (2*S*,7*R*)-2-(*N*-Boc-amino)-7-aminooctanedioic acid, although this was not done for this model system. LL-Diaminosuberic acid ((2*S*,7*S*)-2,7-diaminooctanedioic acid) has been extensively employed as a non-reducible isosteric replacement for cysteine in biologically active peptides.[57-61]

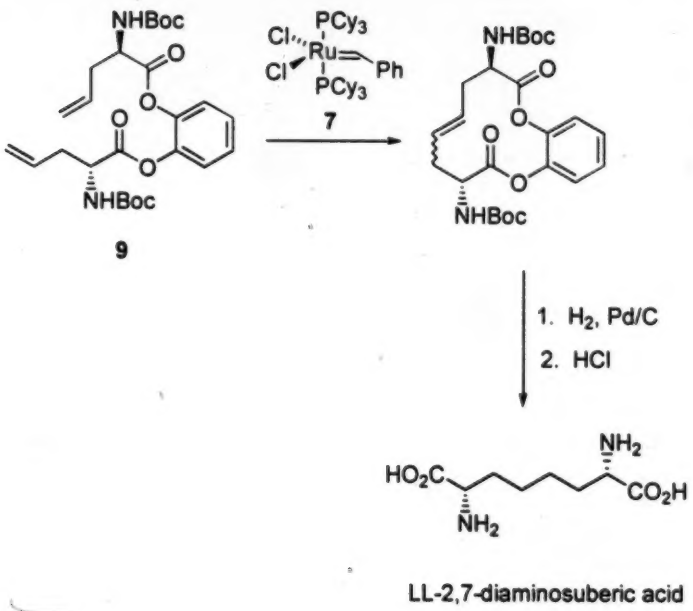
Interestingly, Grubbs' group[62] (California Institute of Technology) and Williams' group[63] (Colorado State University) recently have independently reported similar olefin

metathesis procedures for the generation of chiral 2,7-diaminosuberic acids in high yield. Grubbs' approach (Scheme 5)[62] gives LL-2,7-diaminosuberic acid in 3 steps from acyclic

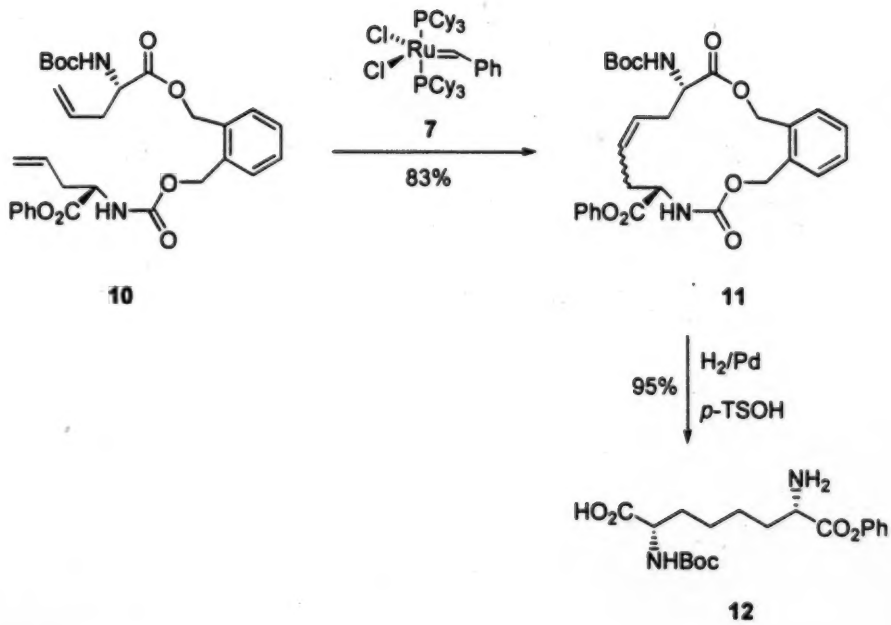


Scheme 4.

diene **9**. The key metathesis cyclization step generates the 12-membered ring in >65% yield. An alternative 14-membered ring **11** was built from **10** (Scheme 6)[63] by Williams and coworkers for macrocylization to differentially protected derivative 2,7-diaminosuberic acid **12**. The higher yields associated with Grubbs' and Williams' methods can be attributed to the fact that two L-allylglycine moieties are anchored to rigid aromatic templates instead of ethylene glycol. Presumably, greater conformation



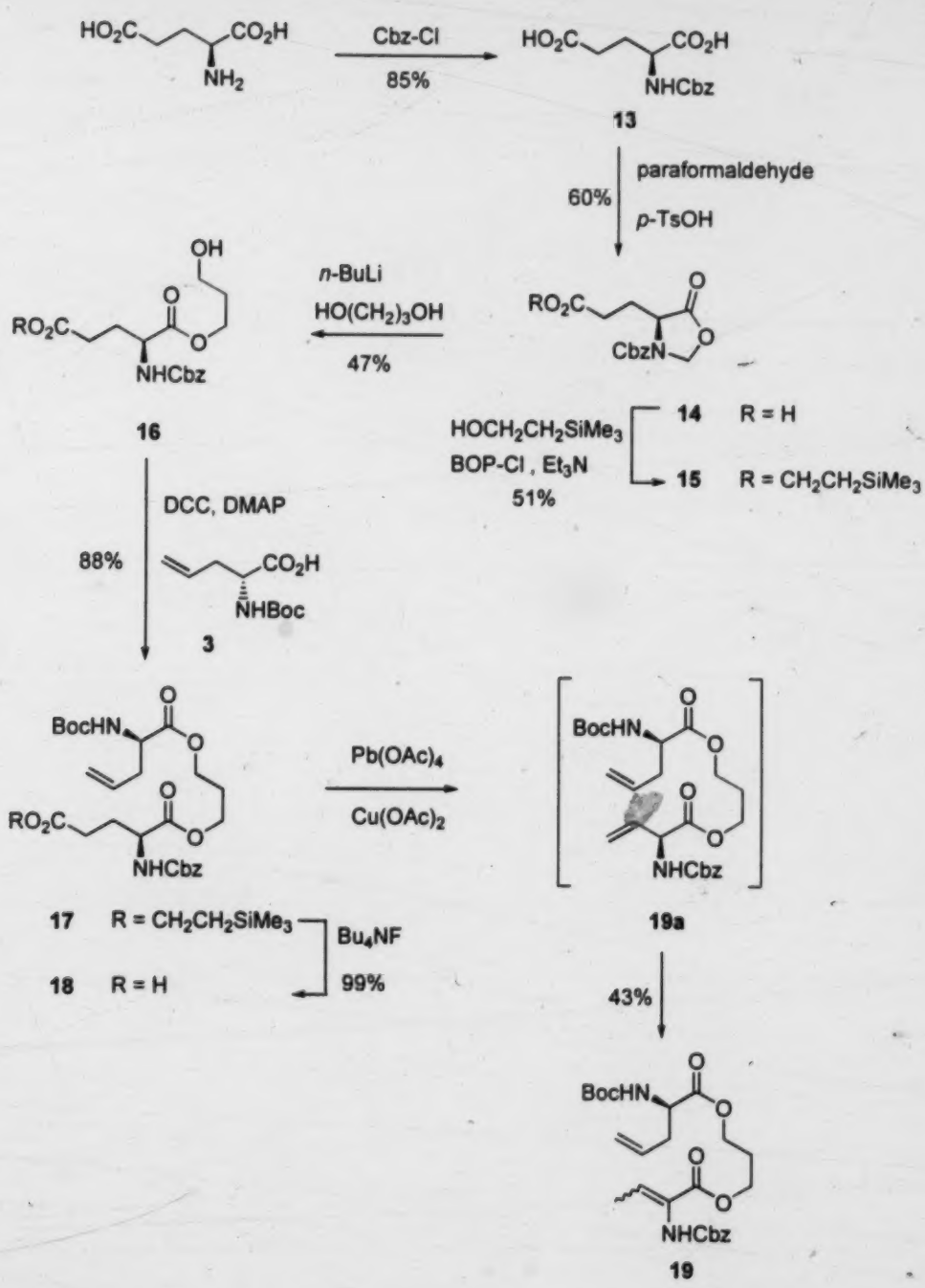
Scheme 5.



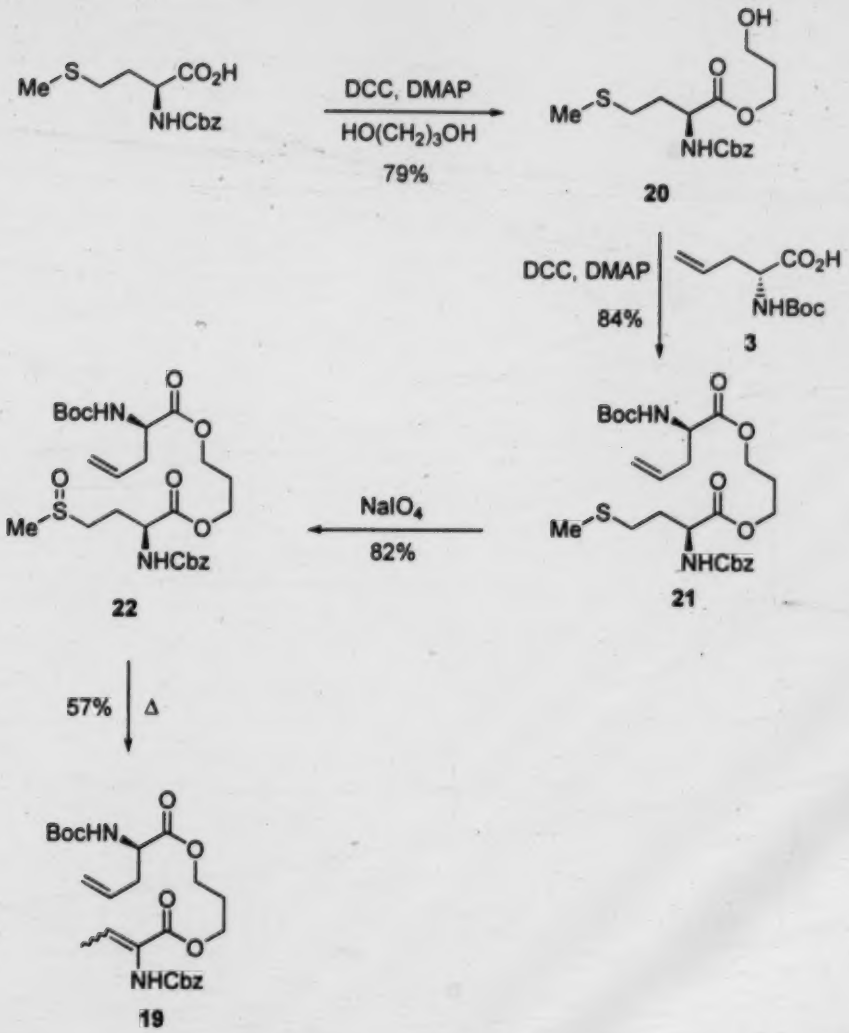
Scheme 6.

restriction facilitates the ring closure process, although formation of 15-membered rings may also be more favorable. Our model study was completed in early 1996 and published recently.[53]

Our work suggests that a similar cyclization of the propanediol diester of *N*-Cbz-L-vinylglycine and *N*-Boc-D-allylglycine could produce a differentially-protected DAP derivative. Although *N*-protected vinylglycine esters are quite sensitive to isomerization of the double bond from the β,γ- to the α,β-position, they can be made by lead tetraacetate decarboxylation of glutamate derivatives[64-66] or by elimination of methionine sulfoxides.[67] Both routes were investigated with a goal of generating the vinylglycine moiety (Schemes 7 and 8). L-Glutamic acid was protected by Cbz-Cl followed by condensation with paraformaldehyde to form oxazolidinone **14** (Scheme 7). Conversion of the glutamate derivative **14** to its trimethylsilylethyl ester **15** followed by reaction with 1,3-propanediol affords **16**, which is readily linked to *N*-Boc-D-allylglycine (**3**) to give **17**. Deprotection quantitatively generates the acid **18**. Unfortunately attempts to generate the vinylglycine moiety *via* lead tetraacetate decarboxylation[64-66] of **18** produce only the conjugated compound **19** and none of the desired vinylglycine derivative **19a**. An analogous approach (Scheme 8) provides the propanediol diester **22** of methionine sulfoxide (54% overall yield for three steps from *N*-Cbz-L-methionine). However, attempted elimination under conditions (195-200 °C, 0.1-0.3 mmHg pressure) known to normally give vinylglycine esters[67] again generates the undesired conjugated **19** rather than **19a.** The reason for the unexpectedly rapid isomerization of presumed intermediate


HO2C
CO2H
NH2
Cbz-Cl
85%
HO2C
CO2H
NHCbz
13
paraformaldehyde
60%
p-TsOH
RO2C
CbzN
n-BuLi
HO(CH2)3OH
47%
OH
RO2C
NHCbz
16
HOCH2CH2SiMe3
BOP-Cl , Et3N
51%
14 R = H
15 R = CH2CH2SiMe3
DCC, DMAP
88%
CO2H
NHBoc
3
BocHN
RO2C
NHCbz
Pb(OAc)4
Cu(OAc)2
BocHN
NHCbz
19a
17 R = CH2CH2SiMe3
18 R = H
Bu4NF
99%
43%
BocHN
NHCbz
19

Scheme 7.

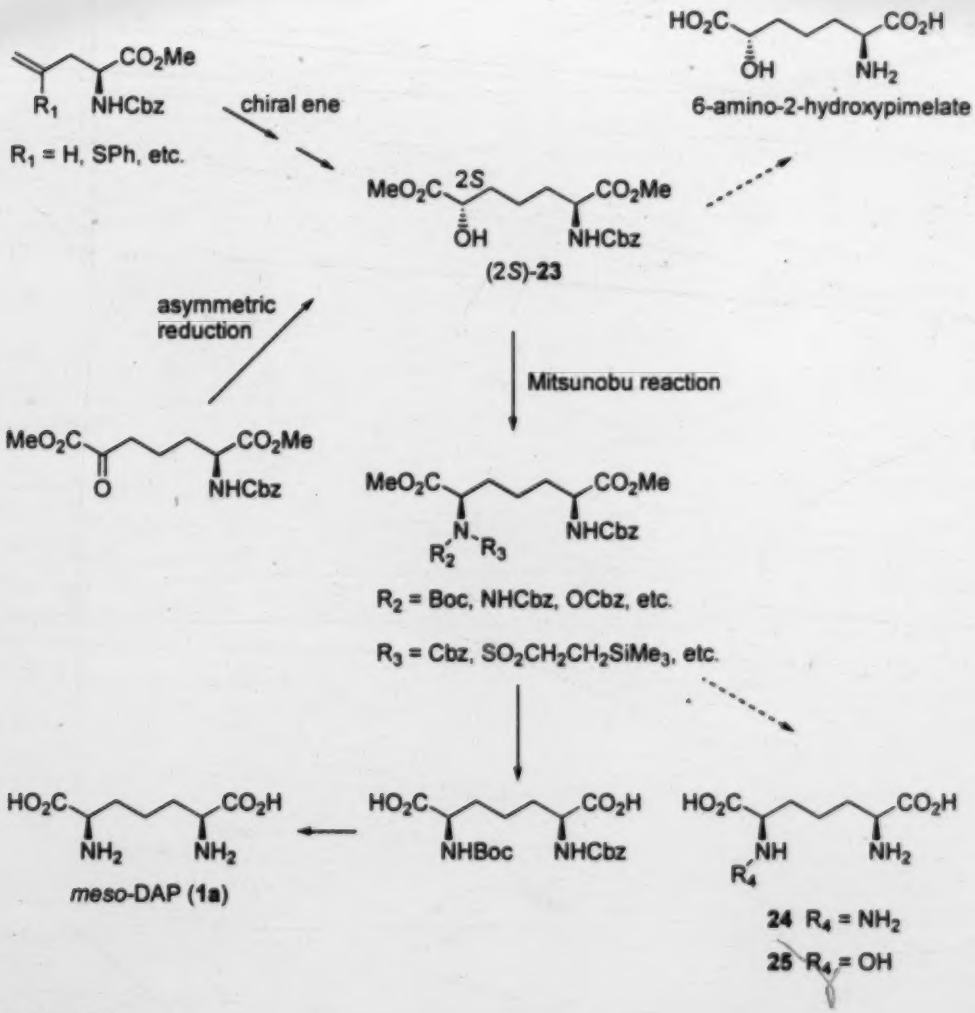


Scheme 8.

19a is unknown, but may involve deprotonation at the α-carbon by a remote functional group in the allylglycine moiety. Nevertheless, the formation of **8** indicates that this approach could be a viable route to a number of compounds bearing orthogonally protected amino acid moieties at opposite ends of a central chain.

Synthesis of DAP Derivatives Using Chiral Catalysts for the Asymmetric Ene Reaction or for Asymmetric Reduction

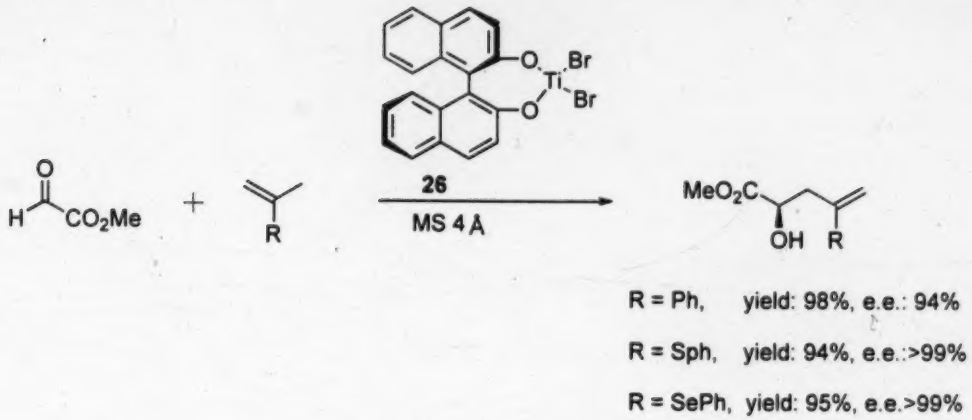
An alternative approach for the assembly of DAP skeleton is the generation of chiral 6-amino-2-hydroxypimelic acid derivative (2*S*)-**23** (Scheme 9), by asymmetric



Scheme 9.

hydrogenation or chiral ene reaction followed by substitution of the hydroxy group with nitrogen *via* Mitsunobu reactions. This synthetic route provides 6-amino-2-hydroxypimelate derivatives as intermediates, some of which have inhibitory activity towards DAP epimerase, dehydrogenase and decarboxylase.[68,69] This route would also allow the stereospecific generation not only of DAP isomers, but also of a large variety of DAP analogues, some of which in the racemic forms like **24** and **25** have already shown activity against several DAP enzymes.[70]

Our first route for stereospecific synthesis of 6-amino-2-hydroxypimelic acid derivative (2*S*)-**23** employs an asymmetric ene reaction[71] as a key step. Recent reports[72-75] by Mikami and coworkers indicate that the chiral titanium binaphthol complex **26** can catalyze ene condensations of methyl glyoxylate and 2,2-disubstituted alkenes to generate α-hydroxy esters with excellent stereoselectivity (94 - 99% e.e.) (Scheme 10). These results, especially those using 2-phenylthiopropene and 2-phenylselenopropene,[75] suggest that catalyst **26** could accomplish stereoselective condensation of methyl (*2S*)-*N*-(benzyloxycarbonyl)-4-(phenylthio)allylglycinate (**30**) with methyl glyoxylate (**32**) to give a 6-amino-2-hydroxypimelic acid derivative with a phenylthio group at γ position, which could then be removed to give desired (2*S*)-**23** (Scheme 11).



Scheme 10.

Thus, 2-(phenylthio)-3-chloropropene was generated by adding phenylsulfenyl chloride to a solution of allene in THF at -30 °C (Scheme 11) which was transferred *via* a cannula, without further purification, into a THF solution of the anion of (*R*)-Schöllkopf chiral reagent **27**[76,77] generated with one equivalent of *n*-BuLi at -78 °C. This gives a 90% yield of thiol ether **28**, which on hydrolysis (0.4 N TFA, 4 hours) affords L-valine methyl ester and (*S*)-4-(phenylthio)allylglycine methyl ester (**29**). The esters were separated by distillation with the desired product as the residue. This was transformed into the protected (*S*)-allylglycine methyl ester **30** in 78% overall yield by reaction with benzyl chloroformate and pyridine in methylene chloride. The enantiomeric excess has been determined to be ≥ 98% by NMR analysis of corresponding camphamides of **29**.

Attempted conversion of **30** to **33** using chiral binaphthol-titanium complex **26** as a Lewis acid fails and leads only to recovery of starting materials. Model reactions of 2-methylpropene and 2-phenylthiopropene (Scheme 10) with methyl glyoxylate (**32**) by

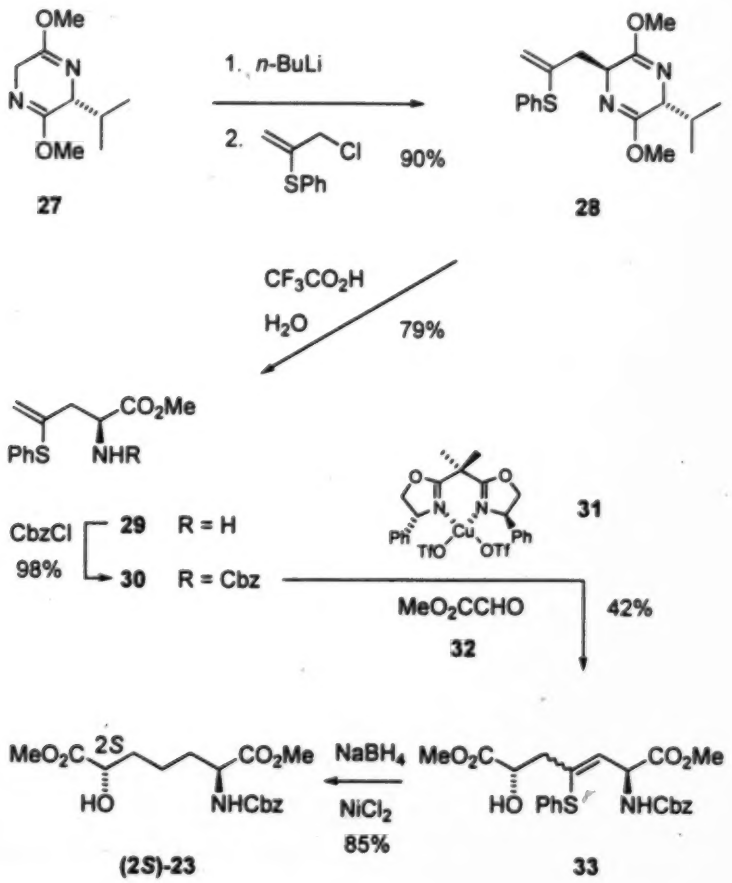
following literature procedures[72] give results consistent with those reported by Mikami's group. This confirms that the binaphthol-titanium catalyst **26** generated in our laboratory is active and the failure of **30** for ene condensation is probably due to the presence of the extra Cbz-protected amino ester moiety. Presumably the amino acid ester can compete with binaphthol or methyl glyoxylate in binding to the titanium reagent, thereby increasing steric bulk or deactivating the alkene.

The bis(oxazoline)-copper complex **31**, which was initially used as a catalyst for asymmetric Diels-Alder reactions[78-80] and was reported to produce undesired ene side products,[81] catalyzes the desired ene reaction between **30** and methyl glyoxylate to give alcohol **33** in a modest yield (42%) (Scheme 11). Attempts to optimize this reaction by replacing copper center with alternative metals, such as nickel, cobalt, iron, zinc, as well as replacement of triflate with chloride, bromide, etc. only lead to a maximum 5% conversion of **30**. Apparently, other bis(oxazoline) complexes are insufficiently active to promote bond formation with the terminal olefin of **30**.

The diastereomeric excess of the newly generated center of **33** could not be easily determined at this stage as this product is somewhat unstable. The olefinic bond and phenylthio group on alcohol **33** are simultaneously removed by Ni_2B (Scheme 11) in a mixture of methanol and THF to generate the saturated alcohol (2*S*)-**23** in 85% yield. Other desulfurization reagents, such as Raney-Ni, tributyltin hydride and lithium in ammonia give primarily undesired over-reduced products.

With desired (2*S*)-**23** available, the next step for *meso*-DAP synthesis is to substitute the hydroxyl group with nitrogen. The method explored previously in our

group (Scheme 3) was the formation of a mesylate by treatment with methanesulfonyl chloride in pyridine, transformation into the corresponding unstable azide, followed by



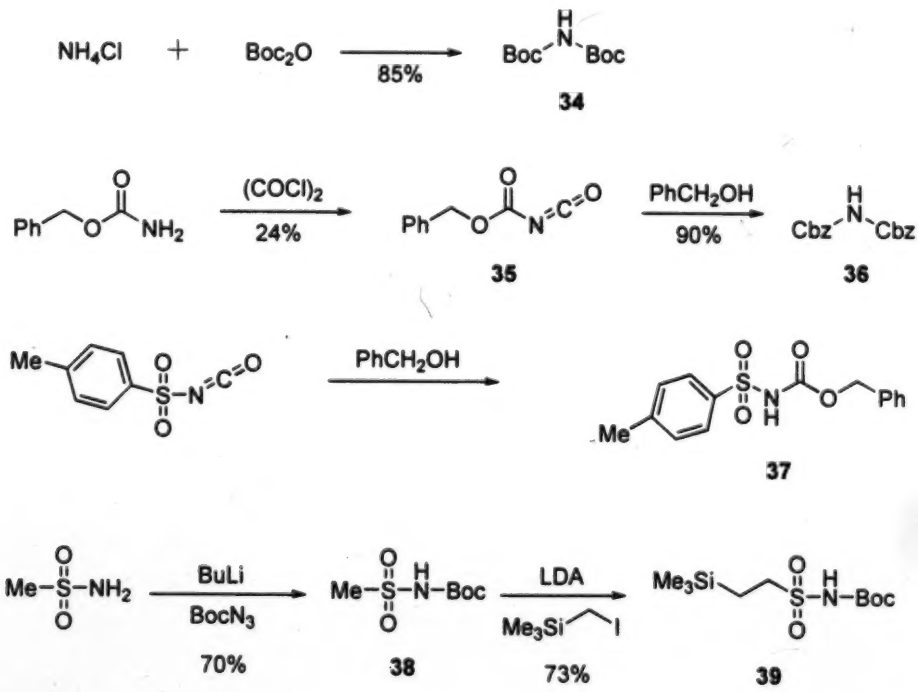
Scheme 11.

hydrogenation. However, this procedure is not satisfactory as the α-hydroxy esters are prone to racemization, especially under very basic conditions. Our new strategy is the hydroxyl substitution using the Mitsunobu reaction which generally introduces nitrogen with 100% inversion of stereochemistry.[82-84] Another advantage of the Mitsunobu

approach would be access to a variety of DAP analogues by use of other nitrogen nucleophiles.

Several doubly-protected amides have been generated and tested as nucleophiles in Mitsunobu processes (Scheme 12). Compounds **34**,[85] **36**[86] and **37**[87] are readily accessible through one or two steps from commercially available materials. Sulfonamide **39**[88] was synthesized by reaction of methanesulfonamide with $BocN_3$, followed by condensation with (iodomethyl)trimethylsilane.

Among these reagents, **39**[88] gives the highest reaction yield for Mitsunobu transformation (Table 2). Thus, the Mitsunobu reaction using amide **39**, diethyl azodicarboxylate (DEAD) and triphenylphosphine in THF converts (2*S*)-**23** to DAP derivative **40** in 79% yield (Scheme 13). This process inverts the chiral center to give a

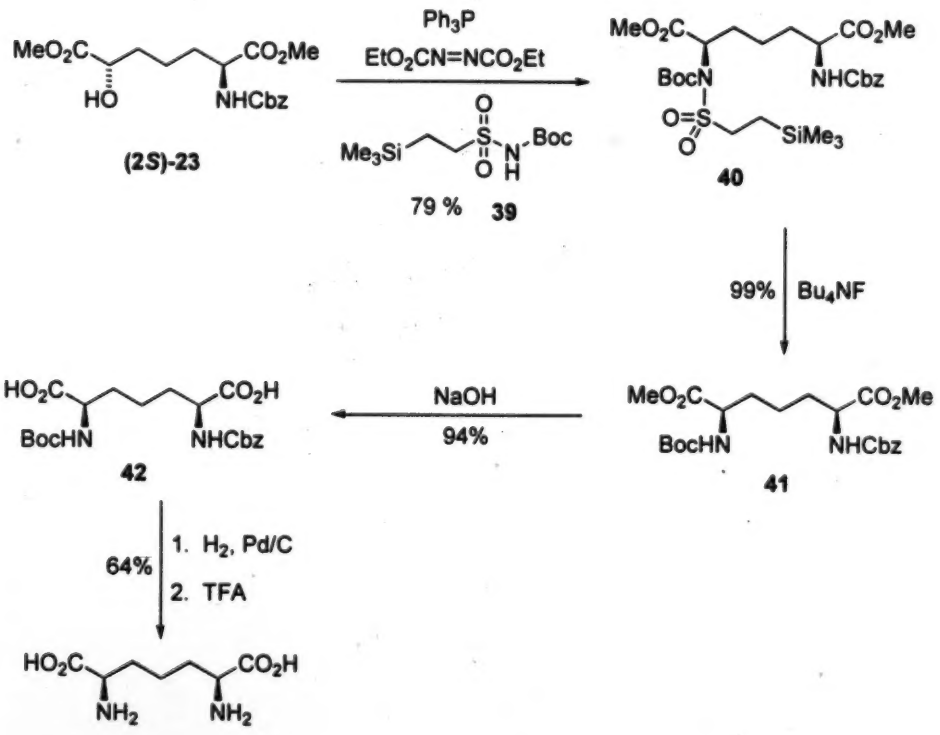


Scheme 12.

Table 2. % Yields of Mitsunobu Reactions between **23** and Doubly-protected Amides

Mitsunobu reagent	% Yield[a]
34	<5
36	<5
37	68
39	79

[a] At 1.5 eq. DEAD, 1.5 eq. amide, 1.64 eq. Ph_3P and 1 eq. **23** in THF at room temperature.



Scheme 13.

DAP skeleton with the desired *meso* stereochemistry. Removal of the silyl protecting group proceeds quantitatively with tetrabutylammonium fluoride in THF to form the fully protected DAP derivative **41**. Saponification of the diester in NaOH solution generates selectively *N*-protected DAP derivative **42** as the desired target compound in 94% yield. The Boc and Cbz protecting groups could then be selectively removed as shown in Scheme 13. Hydrogenation (1 atm) with a 10% Pd/C catalyst followed by acidic removal of the Boc group with TFA/CH_2Cl_2 affords *meso*-DAP **1a** in 64% overall yield. ^{1}H NMR analysis of the corresponding Mosher's diamide derivative (Figure 3) shows the stereochemistry to be 94% (2R, 6S) (**1a**) and 6% (2S, 6S) (**1b**). Since earlier studies show that there is usually no epimerization under the deprotection conditions,[23] and the Mitsunobu process usually occurs with clean inversion,[82-84] this suggests that the key ene reaction proceeds with ca. 88% diastereomeric excess.

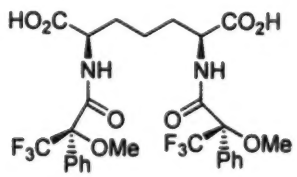
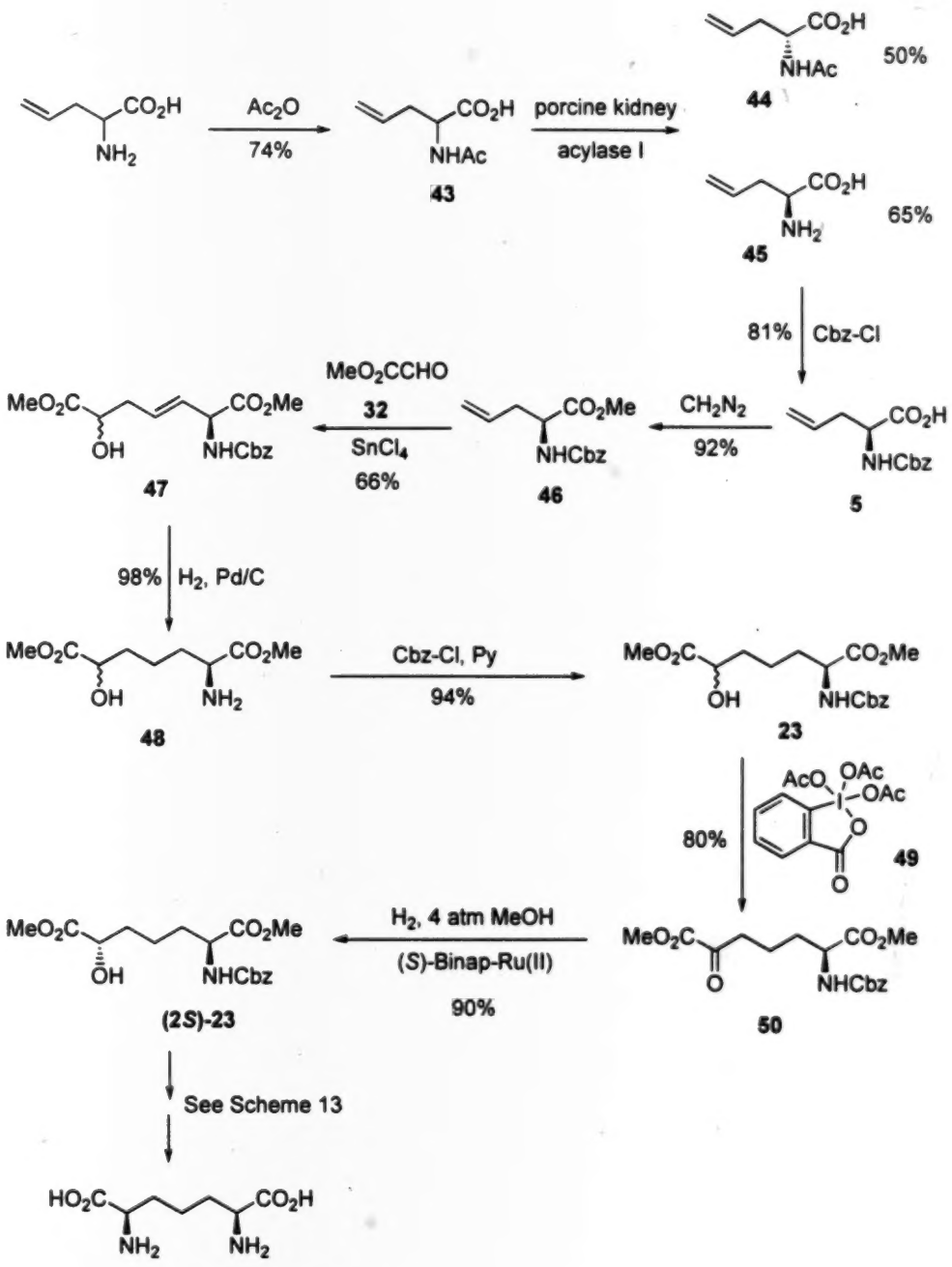


Figure 3. Mosher's Diamide of *meso*-DAP

We also examined an approach to chiral (2*S*)-**23** *via* asymmetric hydrogenation of α-keto ester **50** using Noyori's Binap-Ru catalyst[89] (Scheme 14). Optically pure L-allyl-glycine **45** can be resolved in >99% enantiomeric excess with pork kidney acylase from racemic *N*-acetyl-allylglycine (**43**),[19] which was generated by the condensation of racemic allylglycine and acetic anhydride. Acylation of **45** with Cbz-Cl in aqueous base,


Ac2O
74%
porcine kidney
acylase I
50%
65%
81%
Cbz-Cl
92%
MeO2CCHO
32
SnCl4
66%
98%
H2, Pd/C
Cbz-Cl, Py
94%
80%
H2, 4 atm MeOH
(S)-Binap-Ru(II)
90%
See Scheme 13
79 : 21 meso-DAP (1a) : LL-DAP (1b)

Scheme 14.

followed by methylation with freshly prepared diazomethane generates the ester **46**.[19] A nonselective ene reaction of methyl *N*-(benzyloxycarbonyl)-L-allylglycinate (**46**) and methyl glyoxylate (**32**) catalyzed by tin tetrachloride in anhydrous THF at -23 °C affords the adduct **47** as a 1:1 mixture of diastereomers in 66% yield. Hydrogenation with 10% Pd/C of **47** removes the carbon-carbon double bond as well as the Cbz protecting group to give dimethyl 6-amino-2-hydroxypimelate (**48**). The free amino group was then re-protected by Cbz-Cl and pyridine to afford a saturated hydroxy ester **23**. Oxidation of the hydroxyl group by the Dess-Martin reagent (**49**) generates the α-keto ester **50** in 80% yield. Asymmetric hydrogenation of the keto group of **50** with the (*S*)-binaphthol ruthenium (Binap-Ru) catalyst developed by Noyori *et al.*[89] under 4 atm of H_2 gives a chiral center at the hydroxy position of the desired product (2*S*)-**23** in 90% yield. This product was transformed to *meso*-DAP directly *via* Mitsunobu substitution followed by deprotection reactions as outlined in Scheme 13. NMR analysis after conversion to the bis-Mosher amide indicates that the hydrogenation step gives a 79:21 ratio of diastereomers in favour of the desired (2*S*)-**23** product. Thus, the asymmetric hydrogenation approach is less satisfactory than the previously described ene method using the chiral bis(oxazoline) catalyst for asymmetric induction.

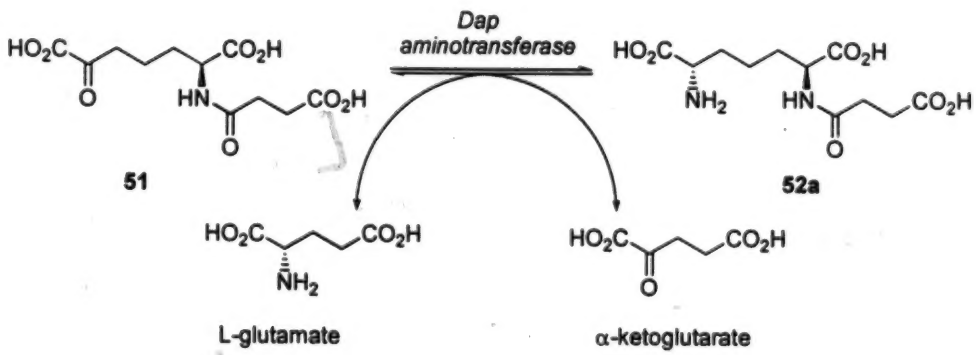
In summary, the stereospecific synthesis of selectively *N*-protected derivatives of *meso*-DAP has been investigated using three approaches: olefin metathesis skeleton assembly, asymmetric ene reaction using chiral copper oxazoline catalysts and stereospecific hydrogenation reduction of an α-keto ester. Although problems with isomerization of vinylglycine moieties blocked metathesis cyclization route to DAP, it is

useful for generation of diaminosuberic acid derivatives and potentially other unusual amino acids. The use of Noyori's catalyst for the stereospecific reduction successfully leads to the desired DAP derivatives albeit with moderate e.e.. The best isomer ratio (94:6) and the most facile method employs a chiral copper catalyst for ene condensation of methyl glyoxylate with **30**. Mitsunobu insertion of nitrogen functionality then affords the targeted DAP derivatives. At this stage, the application of this asymmetric ene approach in the synthesis of DAP analogues of high optical purity is limited only by the availability of chiral ene catalysts suitable for amino acid derivatives. However, this route provides access to a large variety of DAP analogues useful for mechanistic and inhibition studies of DAP-metabolizing enzymes which have very high substrate specificity.

Chapter 3 Mechanistic Studies of DAP Aminotransferase

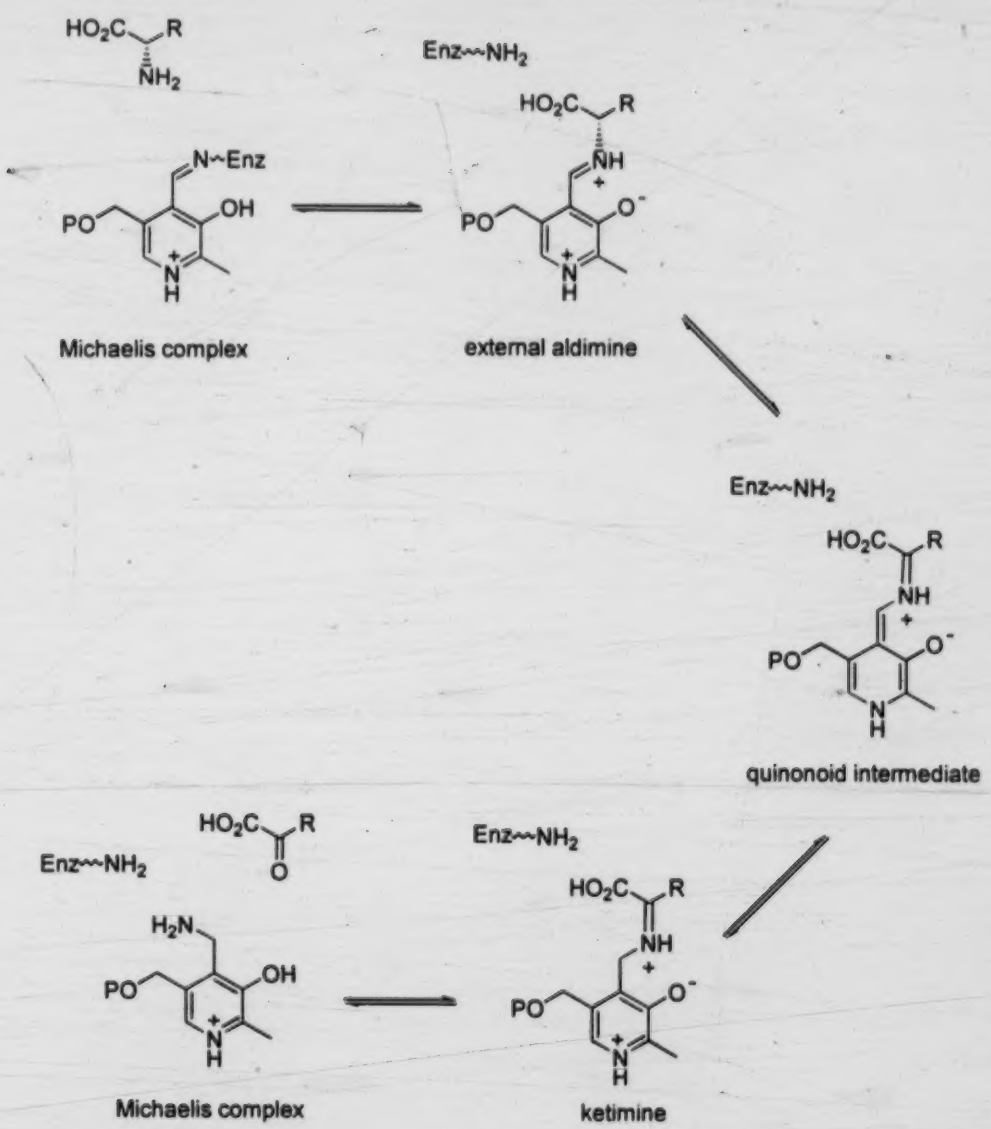
Introduction to Diaminopimelate Aminotransferase (EC 2.6.1.17) (*dapC*)

The *dapC* gene-encoded diaminopimelate aminotransferase (DAP AT) is a pyridoxal phosphate (PLP) dependent enzyme which catalyzes the transfer of the amino group from L-glutamate to *N*-succinyl-L-6-amino-2-ketopimelate (**51**) to generate α-keto-glutarate and *N*-succinyl-LL-diaminopimelate (**52a**)[8,9] (Scheme 15). The Michaelis



Scheme 15.

constants for the natural substrate during DAP amino transfer reaction have been measured: for L-glutamate K_m = 1.21 mM; and for *N*-succinyl-L-6-amino-2-ketopimelate (**51**) K_m = 0.18 mM, k_{cat} = 86 s^{-1}.[19] Kinetic analysis[90,91] also suggests that the transformation proceeds *via* a 'Ping-Pong' sequential mechanism (Scheme 16): L-glutamate reacts with the PLP of the enzyme and donates its amino group *via* aldimine, quinonoid and ketimine intermediates. The substrate (**51**) then



R = $(CH_2)_2CO_2H$ (L-glutamate)

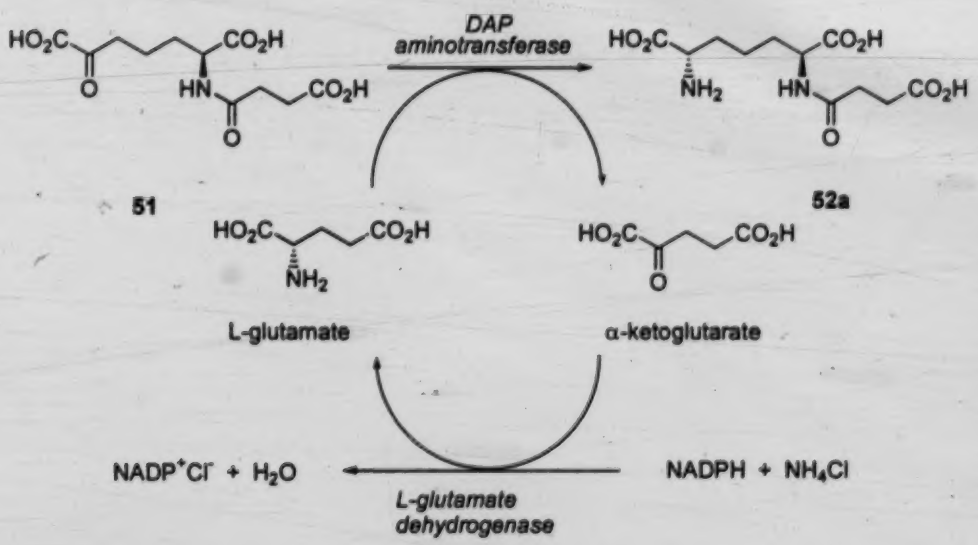
$(CH_2)_3CH(NHCOCH_2CH_2CO_2H)CO_2H$ (*N*-succinyl-LL-DAP)

Scheme 16.

reacts in the reverse direction, regenerating the PLP form of the enzyme with transfer of the amino group onto the product (**52a**). This mechanism is typical of PLP-dependent aminotransferases,[92] consistent with the 'model' of aspartate aminotransferase (EC 2.6.1.1).[93]

Although DAP AT was first partially purified from *E. coli* in 1961,[90,91] relatively few studies have been reported since then. This is mainly due to problems involved with the isolation of the natural substrate **51** from an *E. coli* mutant blocked in deacylase activity. The assay is also inefficient and requires measuring LL-DAP production using ninhydrin in a reaction mixture containing an excess of DAP desuccinylase.[90]

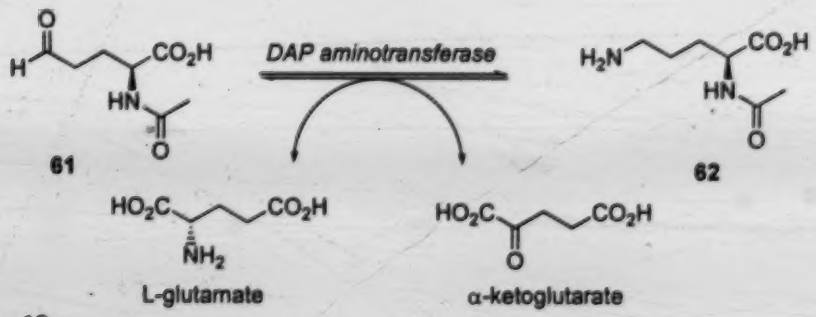
In 1996 our group published a method to synthesize substrate **51** stereospecifically.[19] A more convenient assay was adopted using glutamate dehydrogenase (EC 1.4.1.4) in the presence of NADPH and ammonium ions (Scheme 17)



Scheme 17.

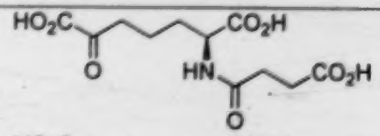
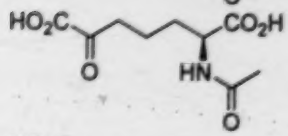
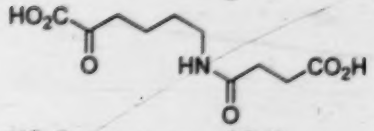
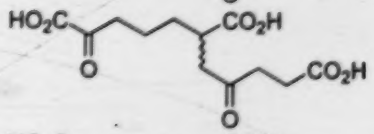
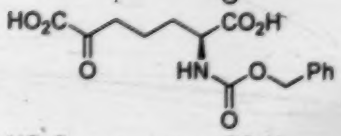
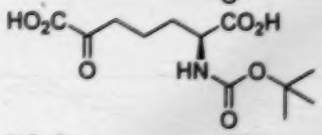
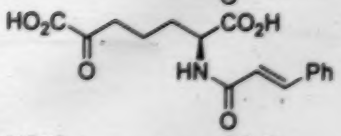
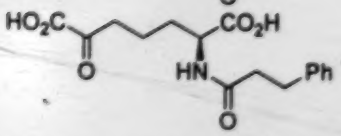
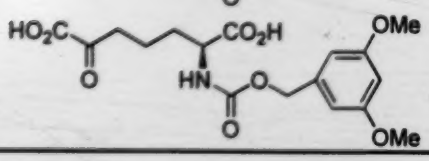
and observing the change of 340 nm absorbance of NADPH. Although DAP AT is specific for L-glutamate, it can tolerate modifications of the acyl side chain of DAP analogues (Table 3). Substitution of the succinyl group by cinnamoyl and Cbz is particularly favored. However, no significant activity could be detected when L-*N*-Cbz-lysine was utilized. This seems to suggest that the terminal C-7 carboxyl group and the DAP 7-carbon skeleton are necessary for enzyme recognition and catalysis, as with other DAP-metabolizing enzymes.

More recently Dr. J. S. Blanchard's group has successfully sequenced the gene of this enzyme from *E. coli*.[94] They found that *dapC* is genetically identical to the *argD* which transforms *N*-acetyl-L-ornithine semialdehyde (**61**) to *N*-acetyl-L-ornithine (**62**) (Scheme 18).[95] Use of synthetic substrates with the purified protein confirms that the same enzyme catalyzes both transformations. These two important amino transfer reactions are done by one *dapC*(*argD*)-encoded enzyme: conversion of *N*-succinyl-L-6-amino-2-ketopimelate (**51**) to *N*-succinyl-LL-diaminopimelate (**52a**) (Scheme 15) and transformation of *N*-acetyl-L-ornithine (**62**) to *N*-acetyl-L-ornithine semialdehyde (**61**) (Scheme 18). Although these two reactions use the same amino donor, L-glutamate, and



Scheme 18.

Table 3. Conversion of Substrate Analogues by DAP AT

no.	structure	K_m^{app} [a]	k_{cat} [b]	k_{cat}/K_m^{app} [c]	k_{cat}/K_m^{app} [d]
51		1.2	86	73	100
53		6.9	23	3.1	4.3
54		0	0	0	0
55		>10	<5	<0.5	<0.7
56		3.6	6.2	17	23
57		8.2	26	3.1	4.3
58		1.2	61	51	70
59		3.5	20	5.6	7.7
60		3.5	76	22	30

[a] unit is mM; [b] unit is s^{-1}; [c] unit is $mM^{-1}s^{-1}$; [c] % rel to **51**.

similar reaction conditions, the substrate *N*-acetyl-L-ornithine (**62**) has quite a different structure than *N*-succinyl-LL-diaminopimelate (**52a**). *N*-Acetyl-L-ornithine (**62**) has only 5-carbons on its main chain with no terminal C-7 carboxyl group.

Mechanistic Studies of DAP Aminotransferase (*dapC*)

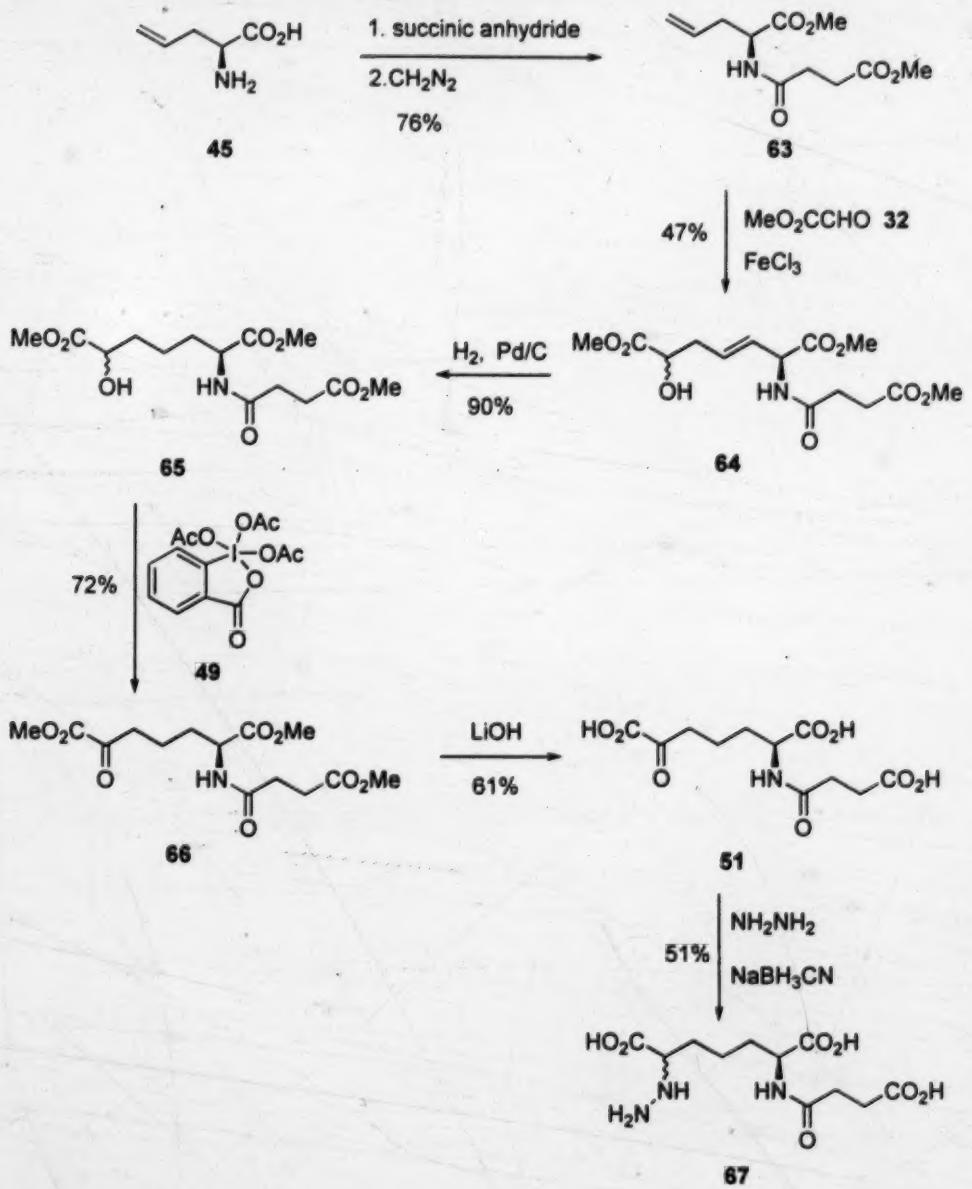
The lack of DAP skeleton in *N*-acetyl-L-ornithine (**62**), a natural substrate for DAP aminotransferase, has generated some interesting questions about this enzyme:

1. How can the enzyme accept these two different substrates? Do **51** and **62** have the same binding site in the enzyme? Do the two substrates take different conformations in the binding site(s) and/or can the enzyme take two different conformations with different substrates?

2. Can the enzyme take other amino acid derivatives as substrates? What are the minimum requirements for recognition?

In order to answer these questions we decided to study this enzyme further *via* two approaches. The first one is to explore the substrate-inhibitor binding site(s) and the enzyme conformation through x-ray crystallographic studies on the enzyme bound to an inhibitor. This work is being done in collaboration with Dr. J. S. Blanchard's group, and two potential inhibitors **67** and **68** were targeted for this purpose (Schemes 19 and 20). Compounds **67** and **68** have hydrazino group instead of amino group, and hydrazino analogues of amino acids are well known as potent inhibitors of PLP-dependent enzymes through formation of hydrazone intermediates.[19,96]

Compound **67** was synthesized (Scheme 19) from L-allylglycine (**45**) using an intermolecular condensation of protected allylglycine **63** with methyl glyoxylate **32** as a

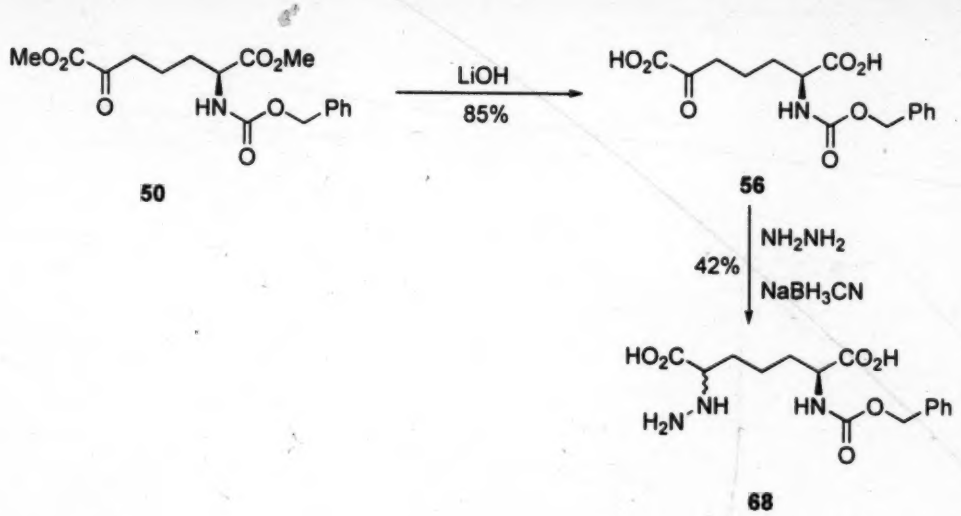


Scheme 19.

key step This procedure is adapted from that developed previously in our group by Dr. Russell J. Cox et al.[19] Acylation of **45** with succinic anhydride in aqueous base, followed

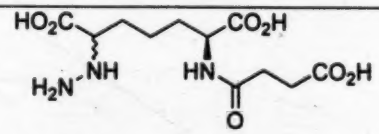
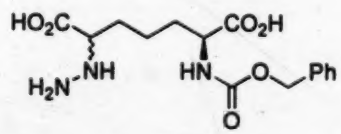
by methyl ester formation with diazomethane provides **63** in 76% overall yield. The ene reaction catalyzed by a Lewis acid ($FeCl_3$) at room temperature affords the ene adduct **64** (1:1 mixture of diastereomers) which is a functionalized α-aminopimelic acid derivative. Catalytic hydrogenation using Pd/C at 1 atm of H_2 proceeds smoothly to give the saturated hydroxy ester **65** in 90% conversion. Dess-Martin periodinate (**49**) readily oxidizes the hydroxy ester to a keto ester **66** (72%). Saponification of **66** with lithium hydroxide monohydrate in water/acetonitrile and then lyophilization generates **51** as a tri-lithium salt. Compound **67** is readily accessible as a 1:1 mixture of diastereomers by treatment of the lithium salt of **51** in methanol with hydrazine monohydrate followed by removal of volatiles and immediate reduction of the crude hydrazone with sodium cyanoborohydride.

A similar method can be applied to generate **68**. Hydrolysis of **50** (from Scheme 14) with lithium hydroxide monohydrate followed by treatment of **56** with hydrazine monohydrate and reaction with sodium cyanoborohydride gives hydrazine compound **68** (Scheme 20). Compounds **67** and **68** are slow tight-binding reversible inhibitors with K_i^* values of 22 and 54 nM respectively (Table 4) for DAP amino transfer reaction against **51**.[19] The $t_{1/2}$ values for release of these inhibitors from the enzyme active site are 79 and 45 min for **67** and **68** respectively. They also completely inhibit overexpressed *E. coli argD* aminotransferase against *N*-acetyl-L-ornithine (**62**) at 50 μM. Although the actual chemical mechanism of inhibition caused by α-hydrazino analogues **67** and **68** is unproven, it is probably similar to that of a number of α-hydrazino acids which inhibit



Scheme 20.

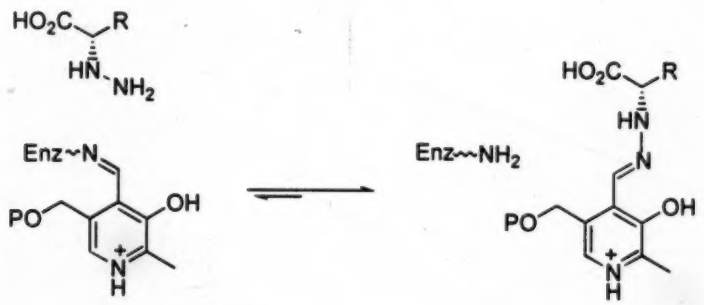
Table 4. Hydrazino Analogues **67** and **68** as Inhibitors of *argD* AT

no.	Structure	*dapC* AT[a] K_i^* (nM)
67		22
68		54

[a] from wild type *E. coli*.[19]

other PLP-dependent enzymes,[96] presumably by formation of a hydrazone with PLP in the active site (Scheme 21). In virtually all PLP-dependent enzymes, the ∈-amino group of an active site lysine forms an imine with the cofactor. This functionality in DAP AT presumably undergoes transamination with the terminal NH_2 group of **67** and **68** to form

the inhibitor-PLP hydrazone. This is probably held by noncovalent interactions in the active site, thereby blocking it. Slow release of the complex and/or hydrolysis of the



Scheme 21.

hydrazone double bond would regenerate the active enzyme. Currently our collaborator, Dr. J. S. Blanchard and his group are working on the crystallization of the enzyme with these two inhibitors. If successful, crystallographic analysis should bring additional insight into the substrate binding by this enzyme.

Our second approach utilizes a number of amino acid analogues (which are divided into four groups, I to IV) (Figure 4) to explore the enzyme's binding/recognition site and mechanism. Group I contains amino acids with 2,4-diaminobutyric acid (4 carbons) as the backbone, group II with ornithine (5 carbons), group III with lysine (6 carbons) and group IV with DAP (7 carbons) skeleton. In each group we used succinyl, Cbz- and acetyl as the protecting groups for the α-amino functionality, since the enzyme can recognize Cbz- and succinyl on the side chain for compounds with the DAP skeleton as well as the acetyl for the ornithine skeleton. It seemed that compound **82**[97] in group IV could be a good analogue since the pKa of hydroxy is similar to that of a protected amide,

which may lead to recognition by the enzyme. It was also proposed that the oximine compounds **77**, **78** and **79** in group IV could mimic the planar character of the α-carbon of quinonoid and ketimine transition states of the substrate-PLP complexes (Scheme 16).

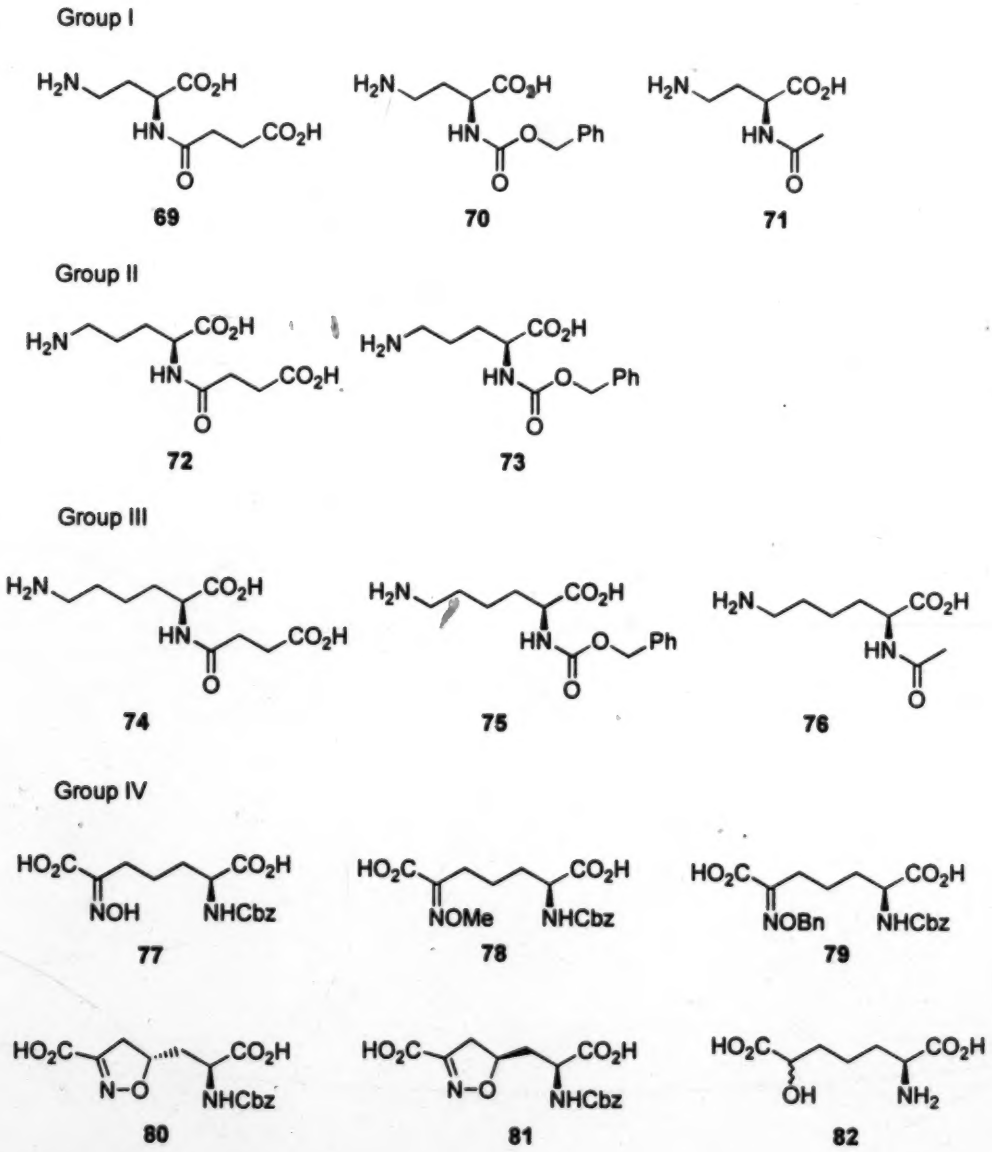
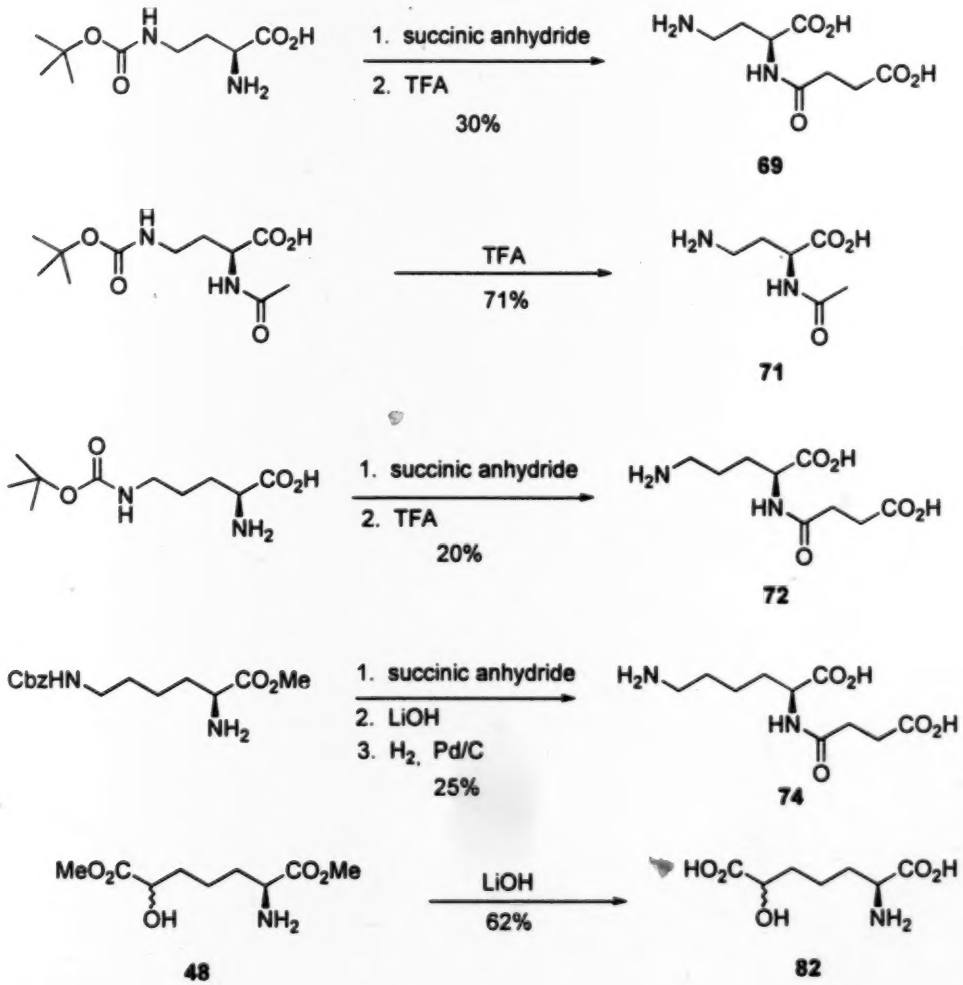


Figure 4. Compounds Targeting *argD* AT

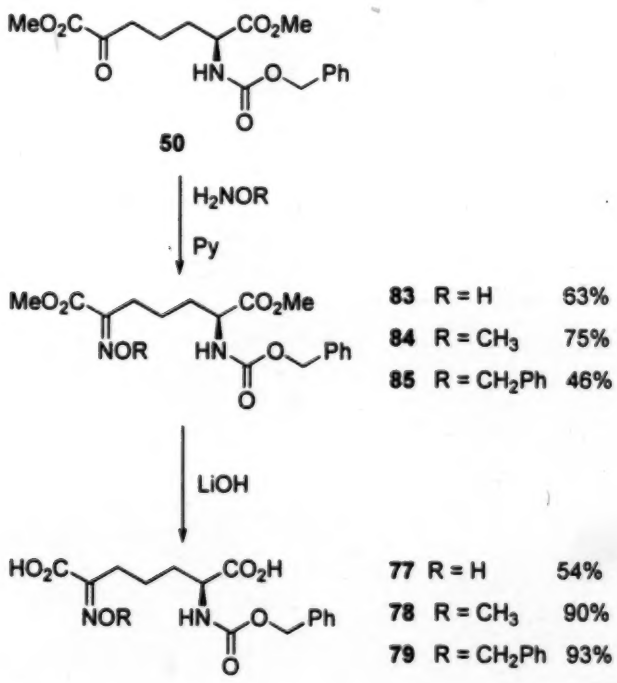
Similarly, isoxazolines **80** and **81** in group IV also contain sp^2 planar centers. In addition, these compounds are available from work on DAP epimerase and dehydrogenase, which is described in the next chapter.

Compounds **69**[98], **72**[99] and **74**[100] (Scheme 22) are readily accessible through acylation of the α-amino groups with succinic anhydride. Without further purification, a



Scheme 22.

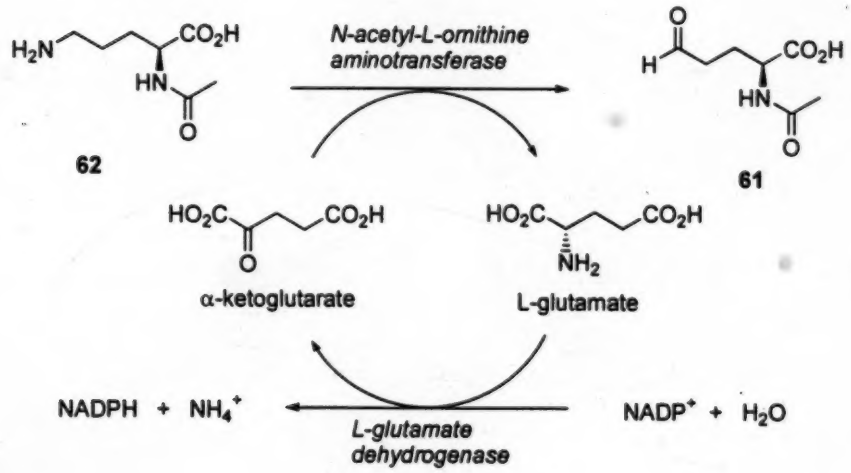
deprotection step was employed to remove either Boc or Cbz groups. Compound **71**[101] was simply prepared from its commercially available precursor *via* removal of the Boc group. Oxime compounds **77**, **78** and **79** in group IV were synthesized (Scheme 23) by condensation of α-keto ester **50** (from Scheme 14) with hydroxylamines NH_2OR (R = H, CH_3, CH_2Ph) using pyridine as the base followed by hydrolysis of the methyl esters. The syntheses of isoxazolines **80** and **81** will be described in Scheme 32 in Chapter 4.



Scheme 23.

The preliminary substrate/inhibition tests were done by Mr. Richard Ledwidge in Dr. J. S. Blanchard's group. An *argD* assay was employed (Scheme 24) which coupled the formation of glutamate from α-ketoglutarate by overexpressed *argD* aminotransferase

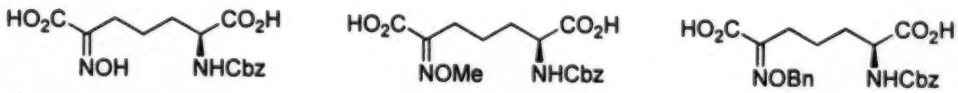
from *E. coli* with excess glutamate dehydrogenase at pH 8.86. The change in absorbance at 340 nm due to NADPH is monitored. This method is very similar to the methodology developed by our group[19] for DAP aminotransferase reaction (Scheme 17).



Scheme 24.

Kinetic data of group IV compounds reveal that the enzyme active site catalyzing amino transfer has a preference for certain structures (Figure 5). Oximine **77** with a hydrophilic OH and a Cbz protecting group at the distal L-center shows 13% and 21% inhibition at 50 and 100 μM, respectively. The hydrophobic OCH_3 derivative **78** gives no detectable activity even at 100 μM level. However, the hydrophobic OCH_2Ph oximine **79** with an aromatic ring exhibits 8% and 17% inhibition at the concentrations of 50 μM and 100 μM, respectively, which is only slightly less than **77**. This suggests that the enzyme active site prefers hydrophilic and aromatic groups. The latter may form hydrogen bonds

through π-cation interactions[102] or may give favorable interactions with aromatic residues in the active site.



77 **78** **79**

12.6% (50 μM) 0% (100 μM) 8.1% (50 μM)

20.7% (100 μM) 16.7% (100 μM)

Figure 5. Oximes against Overexpressed *argD* AT

X-ray crystallographic studies on another PLP-dependent enzyme, aspartate aminotransferase (EC 2.6.1.1), show that a large number of polar and aromatic groups, like Tyr 70, Asp 222, Tyr 225 and Lys 258 are in the active site surrounding the coenzyme PLP (Figure 6).[93] The homology of *argD* encoded protein from *E. coli* and the aspartate aminotransferase from chicken mitochondria has been well established. The two enzymes share 30% sequence identity, including 8 invariant residues and 4 key amino acids for enzyme recognition/binding (Table 5).[103-105] These comparison studies also show a number of hydrophilic and aromatic residues in *argD* AT (e.g. Asp 225, Glu 226, Lys 254) which appear in approximately the same region as Asp 222, Tyr 225, etc. of aspartate AT. Presumably these hydrophilic/aromatic residues of DAP AT in the active site of the enzyme have a strong preference for OH and OCH_2Ph functionalities in **77** and **79**.

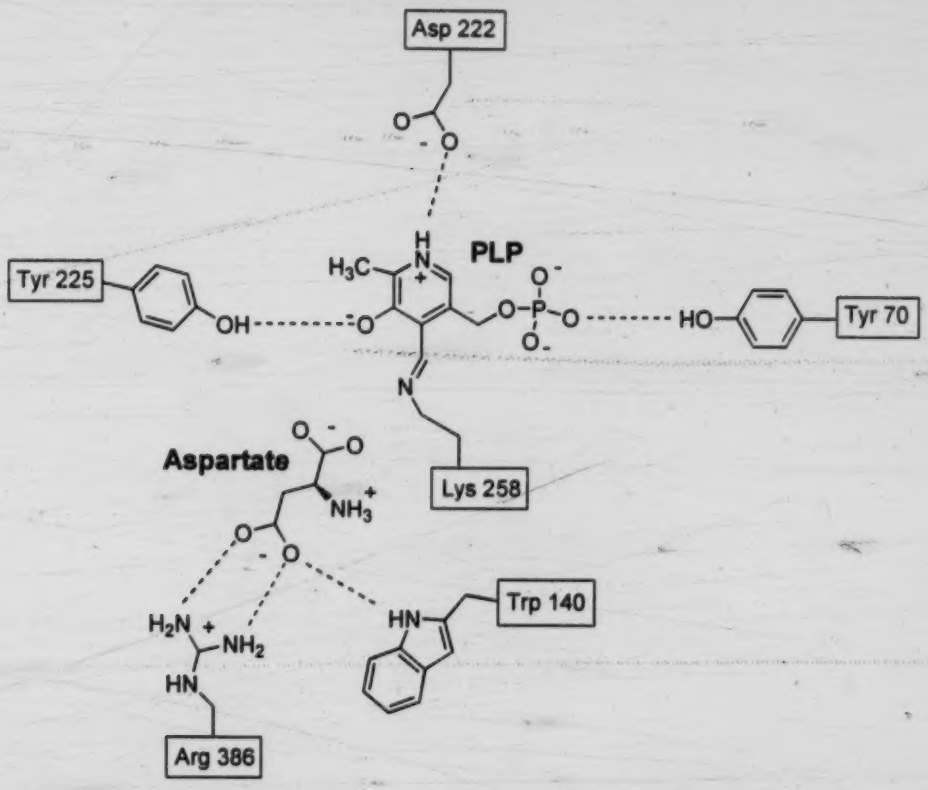
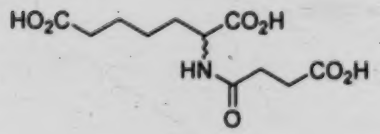


Figure 6. Aspartate, PLP and Key Binding Residues in Aspartate AT

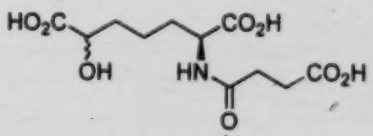
Table 5. Key Invariant Residues in Aspartate AT and *argD* AT

Residue of aspartate AT	Residue of *argD* AT	Specific role in aspartate AT
Asp 222	Asp 225/Glu 226	salt bridge/H-bond to N1 of PLP
Lys 258	Lys 254	Schiff base with PLP
Arg 386	Arg 376	salt bridge/H-bond with α-carboxylate group of substrate aspartate
Gly 197	Gly 199	participates in turn 194-197 located at domain interface

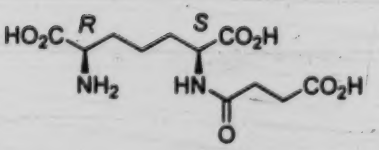
Interestingly, our group found that compounds **86** and **87** (Figure 7) show no detectable effect on DAP AT at concentrations up to 10 mM.[19] Compound **86** is missing the amino group and is not recognized by the enzyme. Alcohol **87** has a polar OH group instead of amino in the natural substrate or oximino (=N-OH) in **77**. Thus, the lack of a basic nitrogen impairs interaction with the enzyme. This indicates that such a nitrogen at the reactive side of the DAP derivatives is necessary for effective substrate recognition by the enzyme.



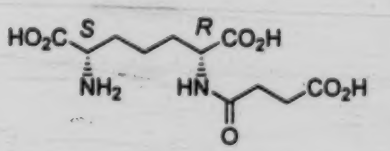
86



87



52b

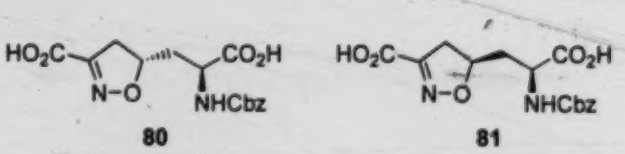


52c

Figure 7. Some Previously Tested Compounds

Previous work has shown that the enzyme is stereochemically specific since the compounds (2*S*, 6*R*)-2-*N*-succinyl-DAP (**52b**) and (2*R*, 6*S*)-2-*N*-succinyl-DAP (**52c**) (Figure 7) possessing a D-configuration at the remote α-carbons show no detectable activity.[19] Interestingly, isoxazoline **80**, which possesses an *S*-configuration at C-5 on the isoxazoline ring, is approximately 4.4 times more active than the corresponding

R-isoxazoline **81** (Figure 8). It may be that the *S*-configuration at the γ position allows **80** to adopt a conformation that imitates the 2-*S* of substrate **52a** when it is bound to the enzyme.



80 **81**

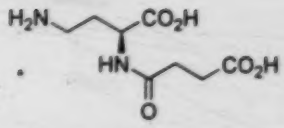
11.7% (50 μM) 2.6% (50 μM)

18.5% (100 μM) 4.3% (100 μM)

Figure 8. Inhibition of *argD* AT by Isoxazolines

Furthermore, an examination of the inhibition results for compounds in groups I - III (Figure 9) reveals that a variety of α-amino protecting groups are accepted by DAP AT. Succinyl protected compounds **69**, **72** and **74** show some interactions regardless of the length of the main amino acid chains: the 4-carbon butyric acid derivative **69** (I) inhibits 20.7% activity of the enzyme at the concentration of 100 μM; the 5-carbon ornithine derivative **72** (II) is slightly less active with 14.5% inhibition at 100 μM level; the least active 6-carbon lysine compound **74** (III) shows 13% inhibition at 100 μM. The ratio of activity at 100 μM among succinyl derivatives **69**, **72** and **74** is 1.6:1.1:1 respectively. However, the acetyl protected **71**, **62** and **76** exhibit remarkably different interactions with the enzyme due to the length of the main chain: 5-carbon *N*-acetyl-L-ornithine (**62**) (II) is a natural substrate for the enzyme, while its 6-carbon version **76** (III)

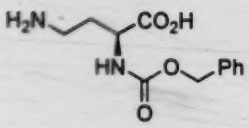
Group I



69

inhibition: 12.2% (50 μM)

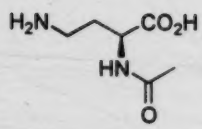
20.7% (100 μM)



70

inhibition: 3.1% (50 μM)

4.6% (100 μM)

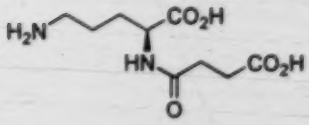


71

inhibition: 1.4% (50 μM)

4.3% (100 μM)

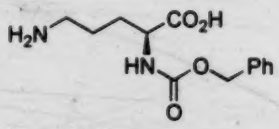
Group II



72

inhibition: 10.5% (50 μM)

14.5% (100 μM)

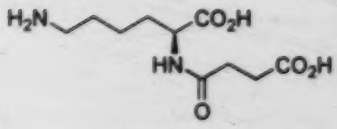


73

substrate activity: 9.0% (50 μM)

19.0% (100 μM)

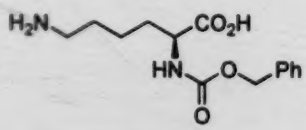
Group III



74

inhibition: 6.6% (50 μM)

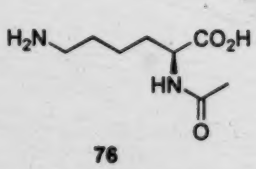
13.0% (100 μM)



75

substrate activity: 0.6% (50 μM)

1.4% (100 μM)

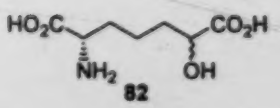


76

substrate activity: 2.9% (50 μM)

5.1% (100 μM)

Group IV



82

inhibition: 6.0% (50 μM)

6.3% (100 μM)

Figure 9. Inhibition of Overexpressed *argD* AT by Group I-IV Inhibitors

is a negligible substrate with only 5.1% activity at 100 μM; **71** (I) is only one carbon shorter than **62** which turns out to be an inhibitor of 4.3% activity at 100 μM concentration. The extension or deletion of one carbon from the natural substrate **62** results in a 23 fold drop in activity. Analogous to the acetyl derivatives, the Cbz-protected amino acid **73** (II) is 14 times more active than its 6-carbon counterpart **75** (III) and 4 times than **70** (I). Compound **73** is an alternative substrate for DAP (*argD*) AT.

The binding site in the enzyme presumably can form hydrogen bonds and other non-covalent interactions with the protected amide and carboxylate of those active compounds in group I-III. This has been observed in aspartate aminotransferase where the substrate aspartate binds to the enzyme *via* hydrogen bonds between the proximal and distal carboxylate group of the substrate and hydrophilic Arg 386 (Figure 6).[93] The Cbz compounds can also bind to the enzyme, possibly through an aromatic π-cation or an aromatic π-stacking interaction. However, the similarity of activity among three succinyl compounds **69**, **72** and **74** has raised some speculations as to the nature of the binding site of the enzyme.

The first explanation is that the succinyl and acetyl compounds, such as **52a** and **62** share one single binding site and this site is quite specific for the lengths of the main amino acid chains. Thus, the 4-carbon and 6-carbon acetyl protected derivatives **71** and **76** are not preferred by this site and only ornithine **62** is accepted. This applies for the Cbz compounds. Succinyl protected amino acids (including natural substrate **52a**) have one extra carboxylate on the amino protecting group which can form additional hydrogen

bonds with the residues in the binding site. This may force the protein to adopt a different conformation to accommodate succinyl compounds.

An alternative hypothesis is that there is more than one binding site for natural substrates **62** and **52a**. The binding site for **62** is more specific than the site for **52a**. Thus, the less specific binding site for **52a** can take a larger variety of succinyl and Cbz compounds. This site is the one which previously has been explored in earlier work by our group.[19] The different binding ability of these two sites is probably due to more and/or larger hydrophobic residues nearby the binding site for **62**.

It is worth noting that the replacement of an amide with OH as in compound **82** only gives a small inhibition of 6.3% at 100 μM (Figure 9), even though the pK_a of an OH group is similar to that of an amide NH. This observation is in accord with the previous report[19] that the lack of an NH group has significantly decreased the activity of **55** (Table 3) compared to the natural substrate **51**. This suggests that the protected amide group may play an important role in the binding/recognition of the substrates by the enzyme. Apparently potential hydrogen bonds between the OH of **82** and residues in the enzyme binding site are insufficient to achieve good recognition by the enzyme.

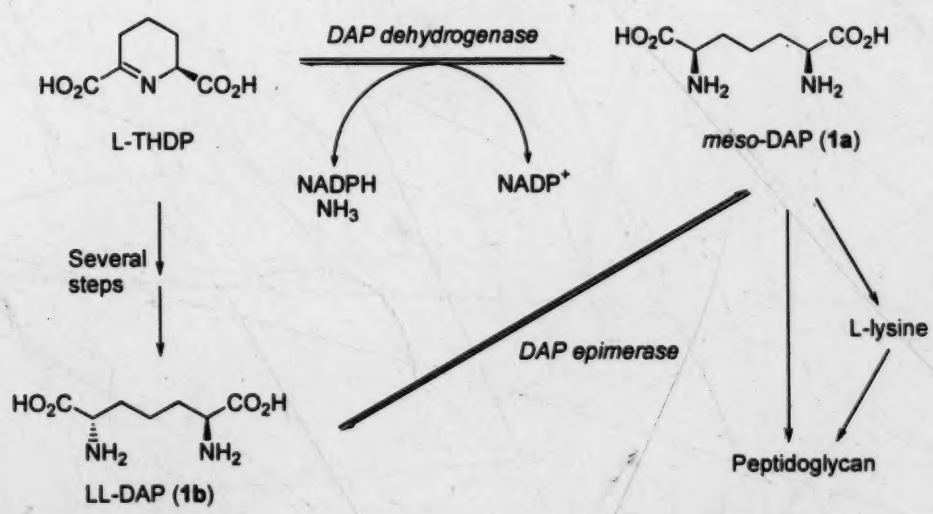
In summary, two hydrazino compounds **67** and **68** have been synthesized and tested against DAP (*argD*) aminotransferase. Further work on the crystallographic studies of the enzyme with those inhibitors is in progress. A number of 4-, 5-, 6- and 7-carbon amino acids have been synthesized and preliminary biological tests with previous knowledge suggest: 1) the active site for the amino transfer reaction has strong stereochemical, and functional group preferences for its substrates or inhibitors; 2) the

nitrogen atom at the reactive side of DAP compounds is necessary for recognition; 3) either one-binding site or two-binding site hypotheses can explain our preliminary data.

Chapter 4 Mechanistic Studies of DAP Epimerase (*dapF*) and Dehydrogenase (*ddh*)

Introduction to Diaminopimelate Epimerase (EC 5.1.1.7) (*dapF*) and Dehydrogenase (EC 1.4.1.16) (*ddh*)

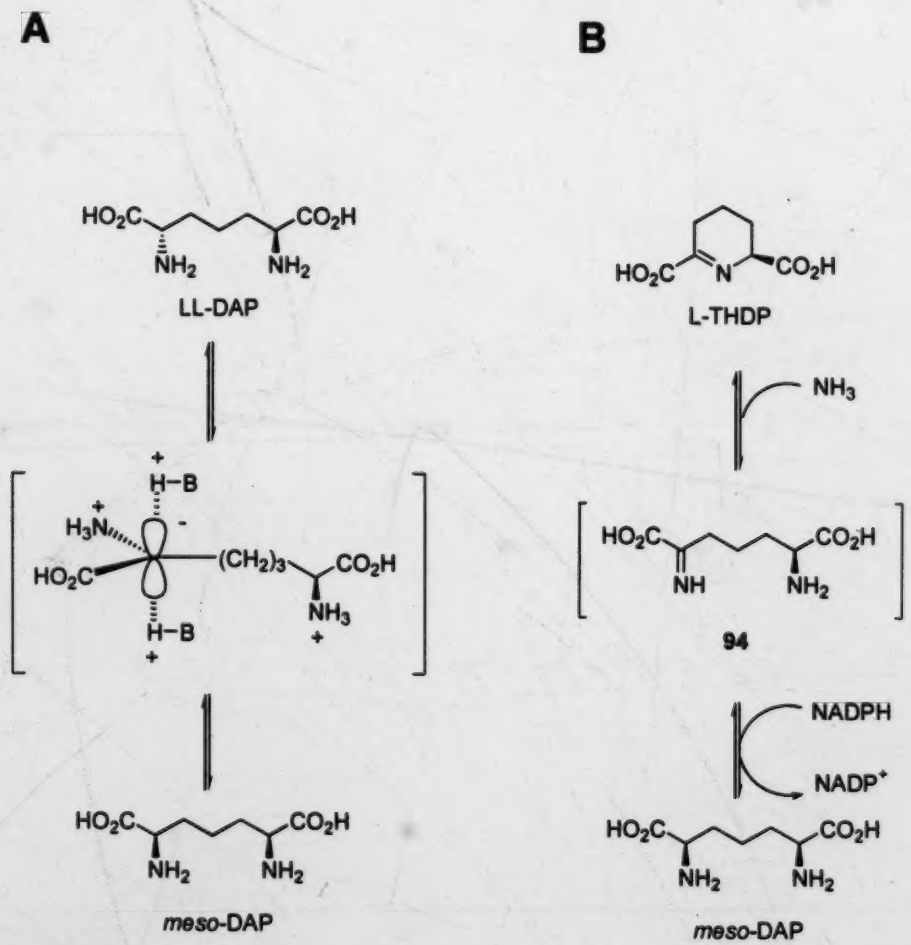
The *dapF*-encoded diaminopimelate epimerase[106-108] catalyzes the interconversion of LL-DAP and *meso*-DAP[109] (Scheme 25) and is a key enzyme for *meso*-DAP biosynthesis.[110] This enzyme has a very strong specificity for substrates. For example,



Scheme 25.

DD-DAP is not a substrate at all. Like proline racemase,[111-113] hydroxyproline racemase,[114] glutamate racemase,[115-118] mandelate racemase[119,120] and aspartate racemase,[121] this enzyme does not require the aid of cofactors, reducible imine, keto functionality or

metals. It may proceed *via* a two-base mechanism (Scheme 26A): one base deprotonates the C-α proton from one face while the second base delivers another proton from the opposite side in a process which gives inversion of stereochemistry at the α-position. Previous studies have shown that Cys 73 probably acts as one of the two bases and the second base is likely to be a different kind of amino acid residue.[108,122] The reaction kinetics for the *meso* to LL-DAP direction give a k_{cat} of 67 s^{-1} and a K_m for



Scheme 26.

meso-DAP of 360 μM; For the reverse reaction, the k_{cat} is 84 s^{-1} and the K_m for LL-DAP is 160 μM.

meso-DAP can also be generated by the *ddh*-encoded diaminopimelate dehydrogenase[123] which converts the intermediate L-tetrahydrodipicolinate (L-THDP) in a single step to *meso*-DAP (Scheme 25).[124] Although DAP dehydrogenase has only been identified in *Bacillus* species,[125,126,133,134] *Brevibacterium* sp.,[127] and *Corynbacterium glutamicum*,[28,128,135] the additional biosynthetic capacity provided by DAP dehydrogenase may allow for increased biosynthesis of *meso*-DAP and L-lysine. This can enhance the ability of such bacteria to survive. DAP dehydrogenase is a NADPH dependent enzyme.[129] For the reductive amination reaction, NADPH binds first, followed by L-THDP and then ammonia. After reaction, *meso*-DAP is released and then $NADP^+$. The mechanism is similar to those previously reported for other amino acid dehydrogenases.[130-132] The reaction presumably proceeds *via* a ring opening of L-THDP by ammonia to give a planar imine intermediate **94** (Scheme 26B). Imine reduction by NADPH then forms the D-center of *meso*-DAP. In the case of the DAP dehydrogenase from *Bacillus sphaericus*, labeling experiments[129] confirm that the *pro-S* hydrogen at C-4 of the dihydronicotinamide ring of NADPH is transferred to the imine intermediate **94** to reduce it to the amino function.

DAP epimerase and dehydrogenase utilize *meso*-DAP as their common substrate/product. The epimerase inverts the D-center of *meso*-DAP to the L configuration and the dehydrogenase oxidizes the D-amino acid center to L-THDP (Scheme 25). The distal unreacted L-center serves as a recognition site for both enzymes. Thus,

compounds having DAP skeletons with small modifications, like **88** - **93** (Figure 10) could be regarded as substrate mimics. These mimics have been synthesized and tested against both enzymes, but they show only moderate inhibitory activity.[24,70]

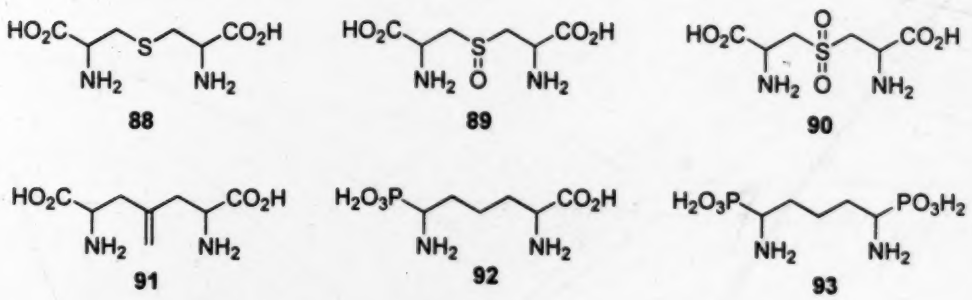
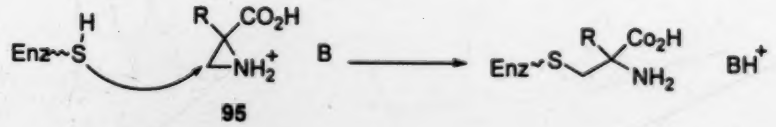


Figure 10. Substrate Mimics of DAP

Another inhibitor of the epimerase that targets its mechanism is more potent. Racemic aziridino-DAP **95** (Figure 11) is an irreversible inactivator of DAP epimerase: opening of the aziridinyl ring by Cys 73 of the enzyme forms a covalent bond which deactivates it (Scheme 27).[122]



R = $(CH_2)_3CHNH_2CO_2H$

Scheme 27.

Compounds **96 - 102** (Figure 11)[17,23,39,70,122,136] either mimic the planar character which develops on the α-carbon of the D-center of *meso*-DAP in the transition state of

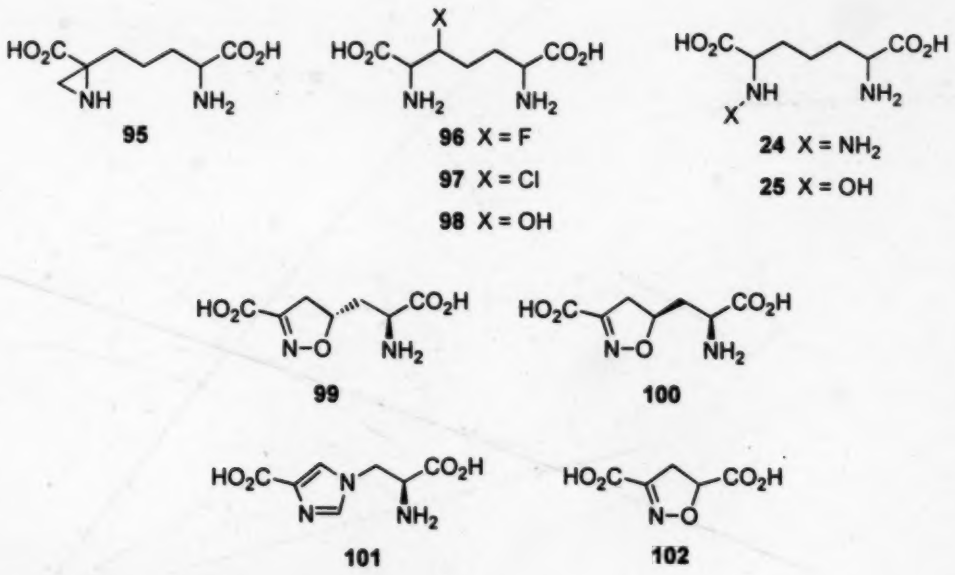
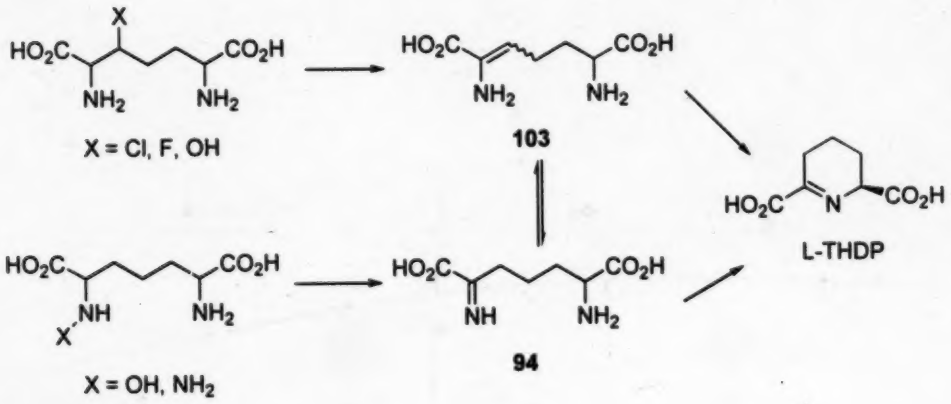


Figure 11. Transition-state Analogues of DAP

the epimerase-catalyzed reaction (Scheme 26A) or by elimination of HF or H_2O can generate such an analogue. Similarly these compounds imitate the imine intermediate **94** in the DAP dehydrogenase reaction (Scheme 26B). Thus, appropriate leaving groups on either the β-carbon (**96**, **97** and **98**) or the α-amino group (**24** and **25**)[17,39,70,136] result in formation of planar transition-state mimics **94** and **103**, which leads to transformation to L-THDP (Scheme 28).[8,17,39,70]

Previous work by Dr. S. D. Abbott in our group[23] has found that although none of the compounds in Figure 11 is active toward DAP epimerase, (2*S*, 5*S*)-isoxazoline **99** is a

potent inhibitor of DAP dehydrogenase. At pH 7.8, it shows competitive inhibition (K_i = 4.2 μM) with L-THDP for the amination reaction and noncompetitive inhibition (K_i = 23 μM) with *meso*-DAP for the reverse process. Preliminary tests for antimicrobial activity



Scheme 28.

demonstrate that **99** inhibits the growth of *B. sphaerius*, which relies exclusively on DAP dehydrogenase to produce *meso*-DAP. In contrast, its diastereomer **100** shows no significant activity toward dehydrogenase at all (13% inhibition at 1 mM). Other heterocycles **101 - 102** with short chains or a different heterocycle do not display potent inhibition even at millimolar concentrations.

Mechanistic Studies of DAP Epimerase

The success of **99** as a potent inhibitor of DAP dehydrogenase[23] has strongly encouraged us to continue exploration of epimerase and dehydrogenase using transition-state mimics, especially isoxazoline-DAP analogues. Thus, isoxazolines **104a** and **104b**

(Figure 12) were targeted as transition-state analogues because both compounds have a planar imine functionality similar to that of **99** and a distal L-amino acid group for recognition. The seven carbon DAP skeleton and the presence of all carboxylic acid groups and basic nitrogens seem essential for enzyme recognition based on previous

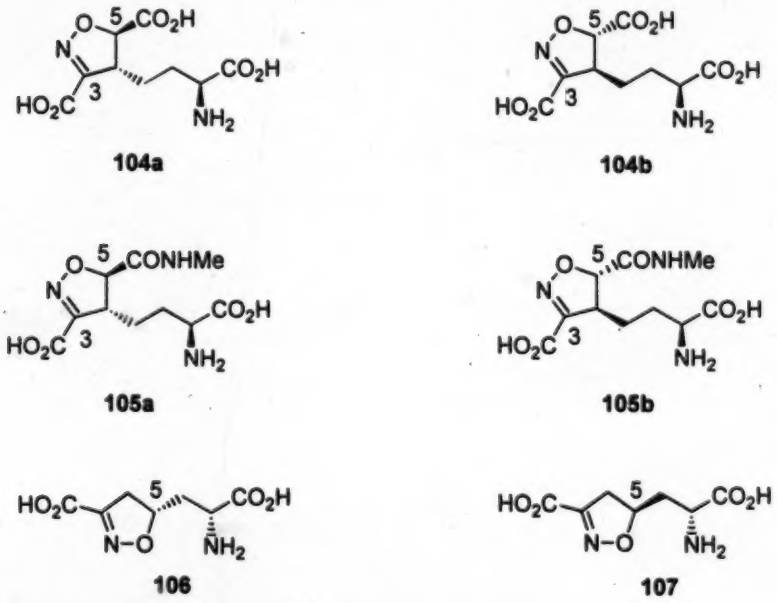
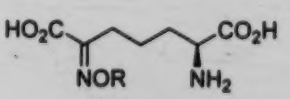


Figure 12. Target Compounds as Transition-state Analogues

results.[52] Unlike **99** which is substituted at the α and γ positions of DAP, isoxazoline rings of **104a** and **104b** are α and β-substituted, which thereby provides an alternative conformation from that of **99**. The carboxylate group at C-5' is essential for facile synthesis by dipolar cycloaddition. It is trans to the methylene substituent and could potentially provide another recognition site because it is seven carbons away from the other carboxyl. Isoxazolines **105a** and **105b** are a protected version of **104a** and **104b**

with *N*-methyl amides instead of the free carboxylates to circumvent extra ionic interactions due to the additional carboxyl. Compounds **106** and **107** are enantiomers of **100** and **99** respectively. Since DAP epimerase and dehydrogenase have recognition sites for both L- and D- centers of *meso*-DAP, the inversion of stereochemistry in the remote site in **106** and **107** may be accepted by enzymes.

Similarly, oximine compounds **108 - 110** (R = H, Me, CH_2Ph) (Figure 13) having no isoxazoline rings were designed as transition-state analogues with a planar oxime functionality. These compounds also have DAP skeletons as well as polar groups for recognition, and the open chain can allow substituents to move freely and assume the correct conformation required by DAP epimerase, in contrast to the heterocycle analogues.



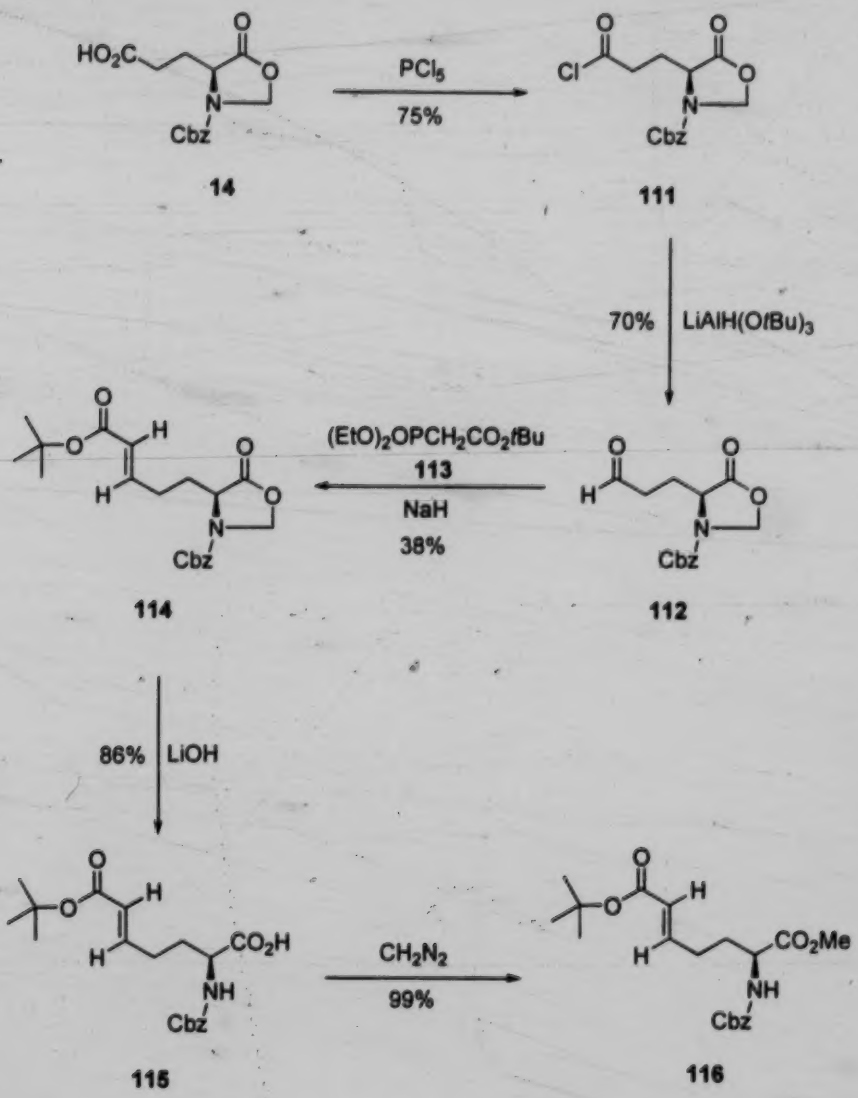
108 R = H

109 R = Me

110 R = CH_2Ph

Figure 13. Oxime Compounds

Compounds **104a**, **104b**, **105a** and **105b** are accessible starting from the partially protected glutamic acid **14**.[154] Reaction of the carboxylate of **14** with PCl_5 (Scheme 29) in benzene at room temperature generates acyl chloride **111** as an amorphous solid in 75% yield.[138] Without further purification, **111** is reduced to an unstable aldehyde **112**[138] by



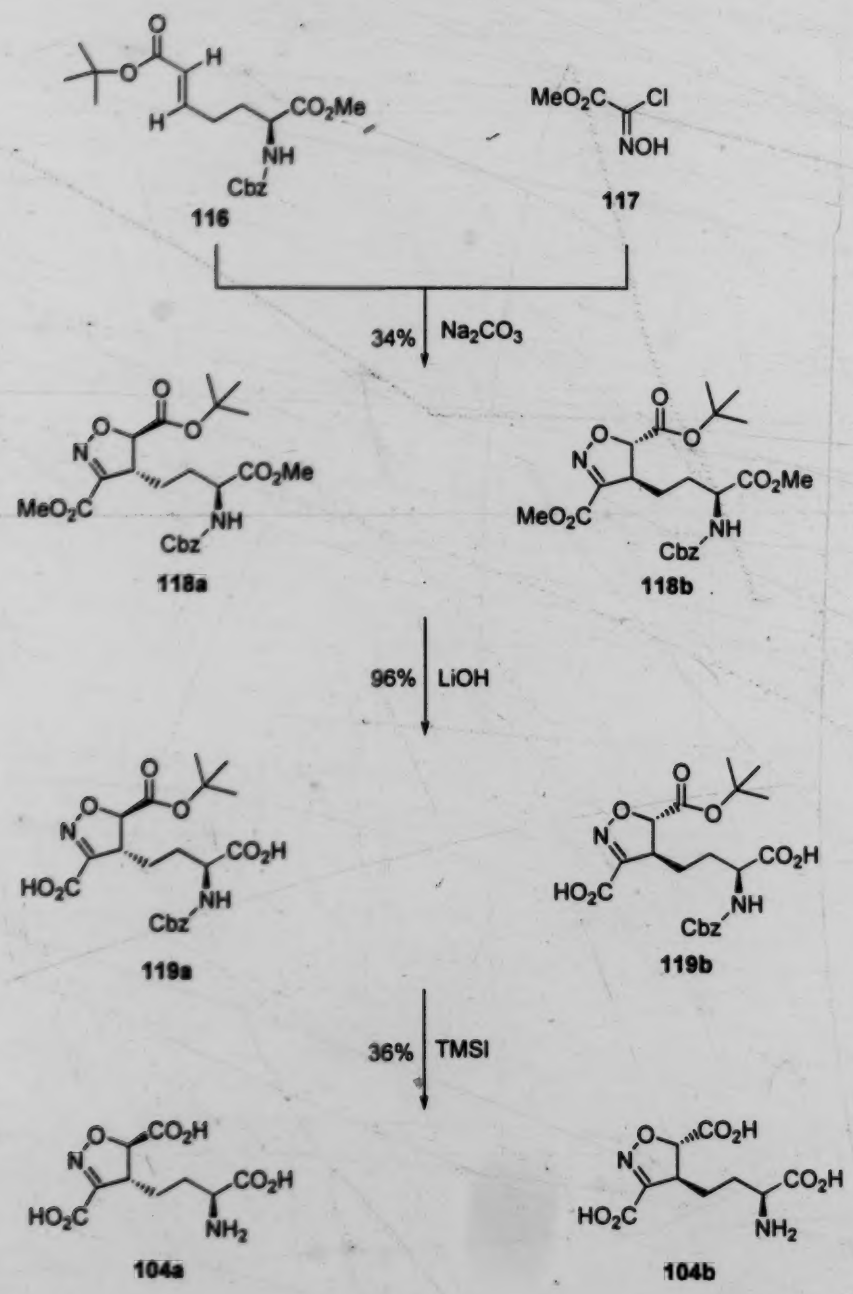
Scheme 29.

the dropwise addition of lithium tri-*tert*-butyloxyaluminum hydride in anhydrous THF at -78 °C. The reaction conditions, especially reaction temperature and the rate of addition

of reducing agent need to be watched carefully, in order to prevent over-reduction of acyl chloride **111** to corresponding alcohol.

The unstable aldehyde **112** can be used without further purification directly in the next step to react with **113** *via* a Horner-Emmons reaction to form the α, β unsaturated ester **114**. The geometry of carbon-carbon double bond can be seen to be trans by ^{1}H NMR spectroscopy since the coupling constant of two olefin hydrogens is 15.6 Hz. Careful saponification of **114** with lithium hydroxide in H_2O/CH_3CN opens the oxazolidinone ring in 86% yield to give the free carboxylate **115**. Esterification of **115** with freshly prepared diazomethane generates **116**.

Compound **116** reacts in a 1,3-dipolar cycloaddition with the nitrile oxide generated *in situ* by base treatment of methyl chlorooximidoacetate (**117**)[23] to give a mixture of **118a** and **118b** (Scheme 30). Although extensive attempts to separate these two compounds for individual analysis were not successful, the regiochemical assignments of the isoxazoline rings of **118a** and **118b** have been determined by ^{1}H NMR using COSY techniques.[151] The spectra show that the oxygen anion of the 1,3-dipole prefers to add to **116** at the α-carbon position of the double bond to give the structures of **118a** and **118b** as expected,[137] since proton correlation between H-4 of the isoxazolines and H-4 of the butanoic acid chains is observed. NOE techniques[151] were used to determine that the relative stereochemistry of the CO_2tBu and methylene substituents on the isoxazoline rings of **118a** and **118b** is trans. Thus, irradiating H-4 of the butanoic acid chains leads to enhancement of the resonances of the isoxazoline rings at H-4 (4.5%) and H-5 (3.3%).

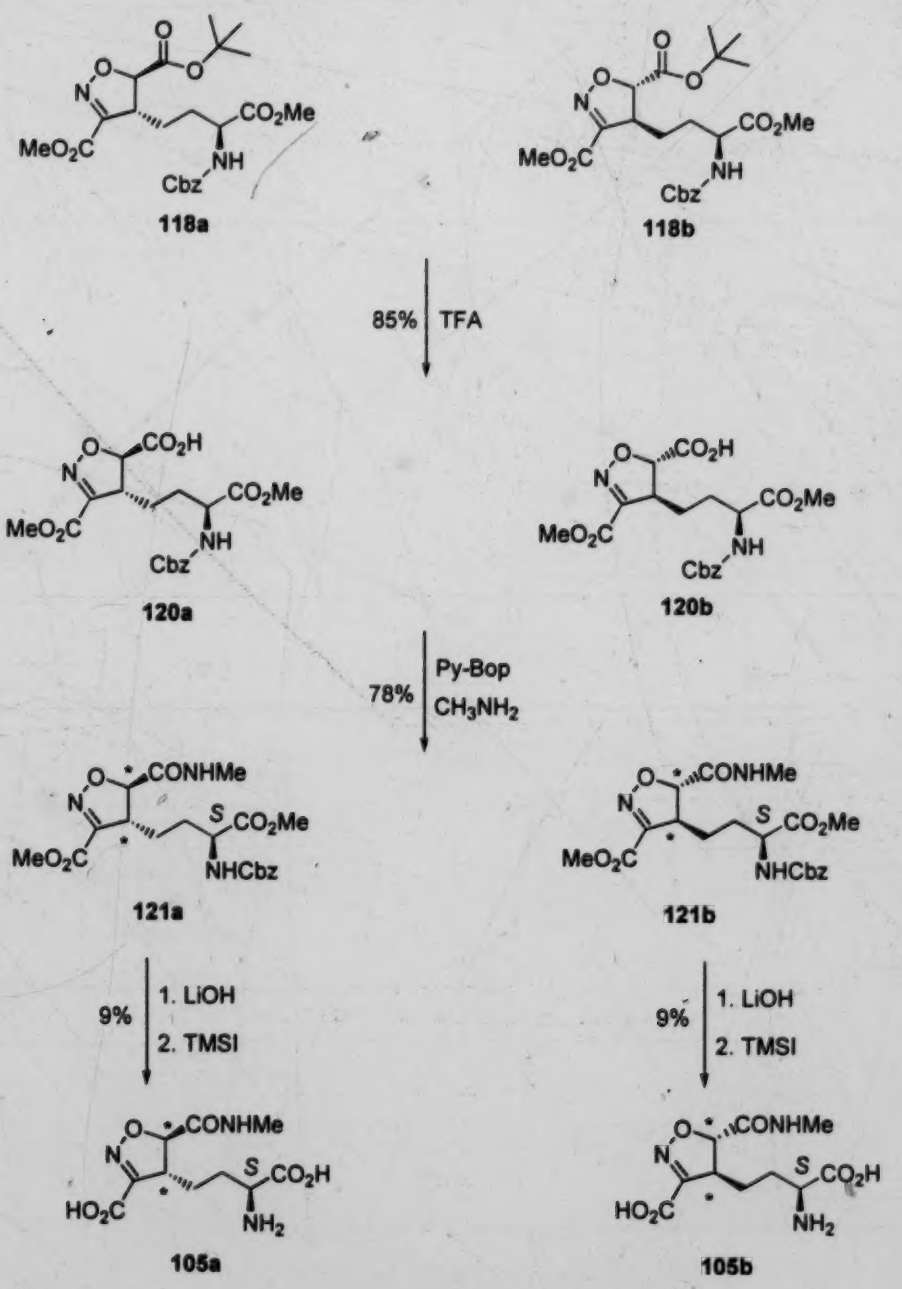

116
117
34% Na2CO3
118a
118b
96% LiOH
119a
119b
36% TMSI
104a
104b

Scheme 30.

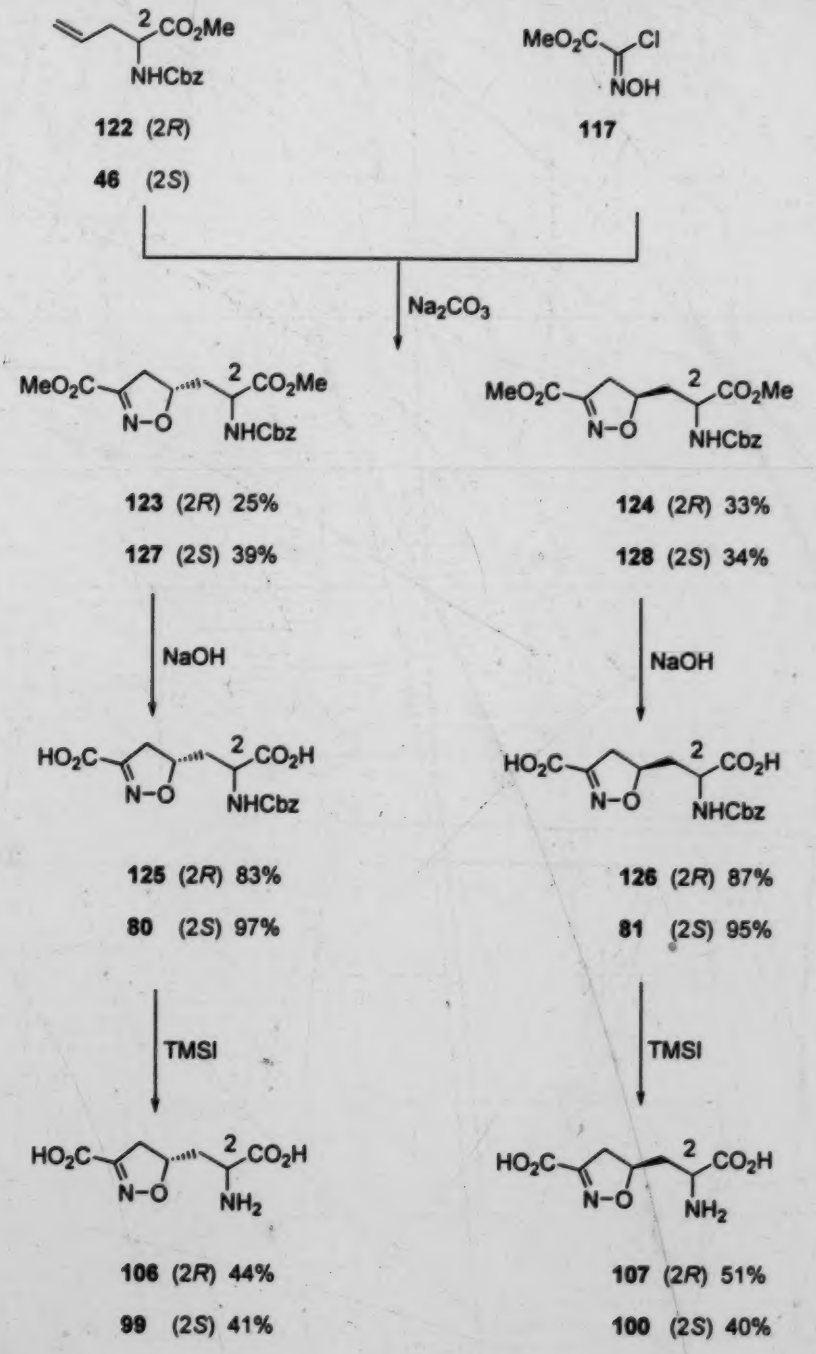
With isoxazolines **118a** and **118b** available, subsequent deprotection steps (Scheme 30) lead to **104a** and **104b**. Hydrolysis of a mixture of **118a** and **118b** with LiOH gives **119a** and **119b** in good yield. Treatment of a mixture of **119a** and **119b** with freshly prepared TMSI allows removal of both Cbz- and *tert*-butyl protecting groups simultaneously to give a mixture of **104a** and **104b** as the desired free amino acids.

Compounds **105a** and **105b** were synthesized as two separated isomers *via* a slightly different approach (Scheme 31). Deprotection of the *tert*-butyl groups of **118a** and **118b** by trifluoroacetic acid (TFA) generates free carboxylic acids **120a** and **120b**. Condensation of the mixture of **120a** and **120b** with methylamine hydrochloride gives amide **121a** and **121b**, which could be separated by flash chromatography. Saponification of **121a** and **121b** followed by immediate deprotection by TMSI generates **105a** and **105b**, respectively, as the desired final products. No attempts were made to determine the absolute configurations of **105a** and **105b** at the C-4 and C-5 of isoxazoline rings, and these compounds were tested directly against DAP epimerase and dehydrogenase.

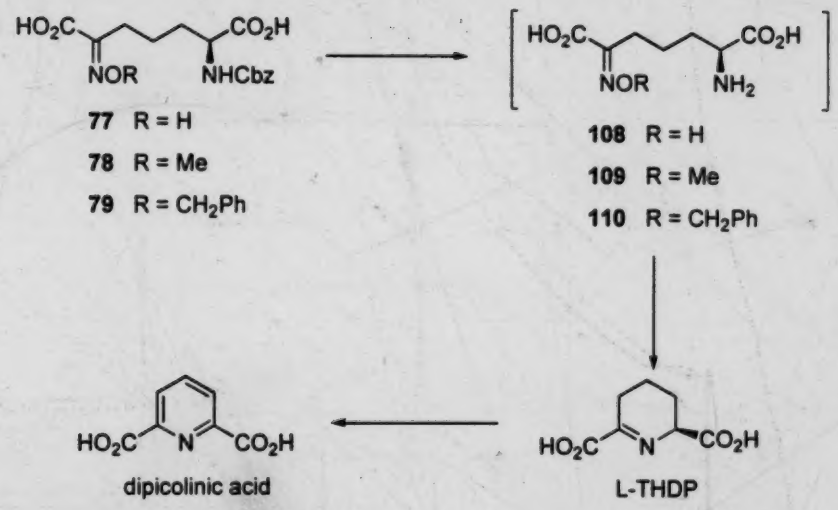
To generate the framework of the isoxazolines of D-isomers **106** and **107**, protected D-allylglycine **122** was condensed with the nitrile oxide derived from **117** (Scheme 32). Two stereoisomers **123** and **124** were separated by flash chromatography and their absolute configurations were determined by comparing the optical rotation values with their enantiomers **128** and **127** which have been previously reported.[23] Saponification followed by deprotection by TMSI generates desired D-isomers **106** and **107** separately. The L-isomers **99** and **100** were re-synthesized separately in a similar manner.


118a
118b
85% TFA
120a
120b
78% Py-Bop
CH3NH2
121a
121b
9% 1. LiOH
2. TMSI
105a
105b
* relative stereochemistry shown may be reverse

Scheme 31.


2 CO2Me
NHCbz
122 (2R)
46 (2S)
MeO2C Cl
NOH
117
Na2CO3
MeO2C
N–O
2 CO2Me
NHCbz
123 (2R) 25%
127 (2S) 39%
124 (2R) 33%
128 (2S) 34%
NaOH
HO2C
2 CO2H
125 (2R) 83%
80 (2S) 97%
126 (2R) 87%
81 (2S) 95%
TMSI
NH2
106 (2R) 44%
99 (2S) 41%
107 (2R) 51%
100 (2S) 40%

Scheme 32.

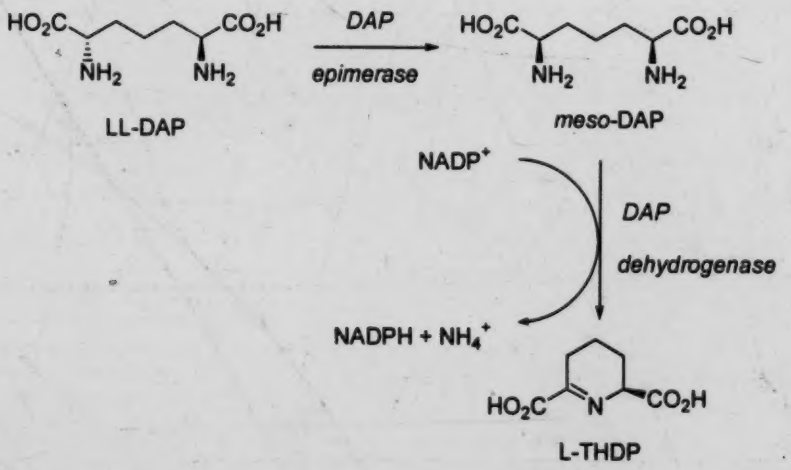


Scheme 33.

The synthesis of the series of compounds **108 - 110** (R = H, Me or CH_2Ph) proved problematic (Scheme 33). Attempts to remove the Cbz protecting groups of **77 - 79** by various means, such as TMSI and hydrogenation gave only trace amounts of **108 - 110** that could be detected by MS and TLC. Subsequent attempts at purification lead to complete decomposition of these materials. Although it is uncertain why oximes **108 - 110** are unstable, one can assume that they cyclize to give unstable L-THDP,[139] which can then oxidize further to dipicolinic acid.

The inhibition tests of **104a**, **104b**, **105a**, **105b**, **106** and **107** with DAP epimerase were done by Ms. Jennifer Caplan in our group using a coupled assay (Scheme 34)[23] with LL-DAP, DAP epimerase and DAP dehydrogenase by monitoring the 340 nm absorbance of NADPH. Substrate tests were conducted by incubating testing compounds in D_2O with epimerase followed by deuterium NMR spectroscopy. However, to our

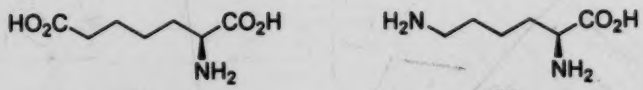
disappointment, none of these compounds shows significant activity even at concentrations up to 5 mM.



Scheme 34.

The failure of isoxazoline compounds to interact with DAP epimerase prompted us to re-consider our strategies on the design of potent inhibitors toward this enzyme. Although reasons as to why these compounds are not inhibitors are not clear at this stage, one can postulate that steric and conformational effects may contribute to the problems. Also the extra carboxylates and methyl amides on the isoxazoline rings of **104a**, **104b**, **105a** and **105b** or even the isoxazoline rings themselves may be too bulky to be accommodated in the epimerase's active site. The fixed isoxazoline rings may also prevent the substituents from adopting the correct conformation to mimic the postulated transition-state geometry recognized by the epimerase.

It is also possible that the DAP epimerase recognizes not only the distal L-center but also the whole amino acid function of the reactive side (D-center of *meso*-DAP). The lack of amino group with an NH in the isoxazolines may eliminate activity of these analogues. In fact, all reported DAP analogues which show strong activity with epimerase (Figure 10 and Figure 11) have either α-primary or secondary amine functions at the reactive side.[17,23,24,39,70,136,140] Our earlier work on the interaction of DAP epimerase with phosphorous **92**[24] and β-fluorodiaminopimelate isomers **96**[39] suggests that the enzyme active site recognizes and binds the amino and carboxylate groups at both ends of the substrate and holds the flexible substrates quite rigidly. More convincingly, previous results in this group by Dr. P. Lane-Bell,[52] show that 2-aminopimelate (**129**) and L-lysine (**130**) (Figure 14) having either a missing amino group or a missing carboxylate are not active at concentrations up to 3 mM.



129 **130**

Figure 14. Previously Tested Compounds

The isoxazolines also lack a hydrogen at the α-center which may prevent effective recognition by the residues in the enzyme active site. Mandelate racemase[119,120] and proline racemase[111-113] utilize a similar two-base mechanism for protonation and deprotonation as DAP epimerase. Replacement of Glu 317 and Lys 166 with Gln and Arg, respectively, results in almost complete loss of activity of mandelate racemase due

to the fact that these are two key residues which form hydrogen bonds to the substrate.[119,120] In the case of proline racemase, pyrrole-2-carboxylic acid (Figure 15) has been suggested to be more tightly bound to the enzyme than the natural substrate proline because of its planar character at the α-carbon.[111] However, 2-furoic acid (Figure 15), which also has an α-planar carbon, is approximately 10 times less active than pyrrole-2-carboxylic acid. Presumably the replacement of NH with oxygen causes the loss of a

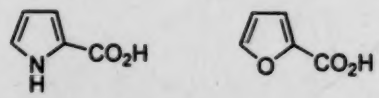


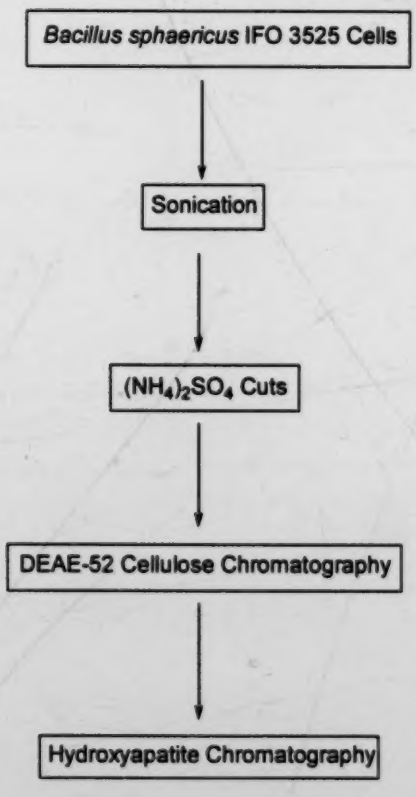
Figure 15. Pyrrole-2-carboxylic Acid and 2-Furoic Acid

necessary hydrogen bond. DAP epimerase has a higher substrate specificity than proline racemase and mandelate racemase, and the hydrogen bonds may play more important roles. The lack of cationic character at neutral pH may also explain why 2-furoic acid[111] (Figure 15) is not as effective as a transition-state inhibitor against proline racemase.

Mechanistic Studies of DAP Dehydrogenase

Our continuous work on DAP dehydrogenase focuses on testing of a number of new transition-state and substrate analogues with this enzyme and x-ray crystallography in a collaboration project with Dr. J. S. Blanchard's group. In order to do the enzyme assay, DAP dehydrogenase was partially purified from *Bacillus sphaericus* IFO 3525 (cultured in Department of Microbiology by Professor Michael Pickard and Mr. Richard Mah). The purification steps used in our study are summarized in Scheme 35.[23,129] Cells are ruptured by sonication and the debris is removed by centrifugation to give a brown

supernatant. Ammonium sulfate cuts (40 and 80% saturation) gives most DAP dehydrogenase activity in the 40-80% pellet and no activity in the final supernatant. The pellet is re-suspended and dialyzed to give a brown solution which is further purified by DEAE-52 cellulose column and hydroxyapatite column. Active fractions are combined and concentrated to a final 50 mL of partially purified DAP dehydrogenase with the activity of 4.84 units/mL and the specific activity of 2.8 units/mg (a unit of activity is 1 μmol/min NADPH formed under assay condition). Incubation with LL-DAP showed no indication of the presence of DAP epimerase contamination in the dehydrogenase solution since the change of 340 nm absorbance of NADPH was not observed under



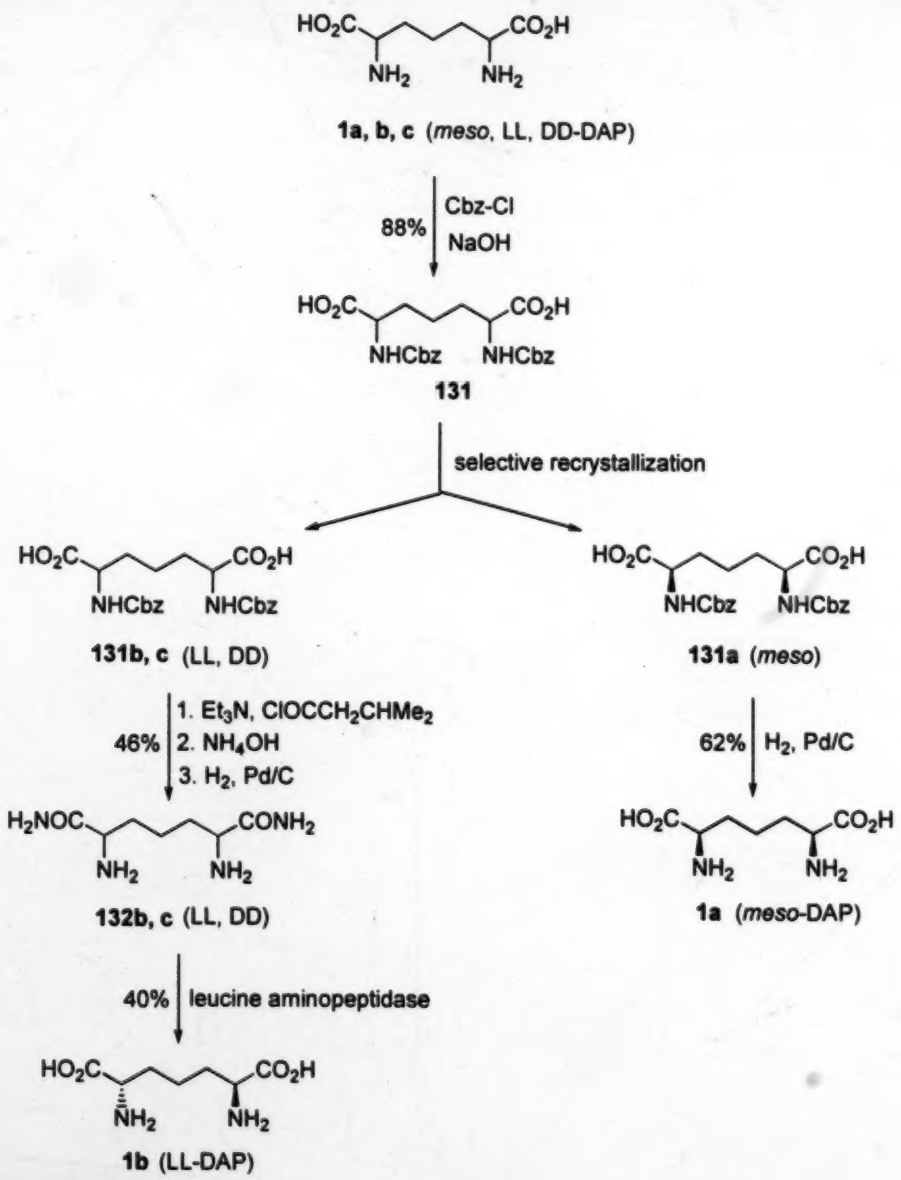
Scheme 35.

assay conditions. DAP epimerase contamination in the DAP dehydrogenase solution would transform LL-DAP to *meso*-DAP, which would then be converted to L-THDP by DAP dehydrogenase with the concomitant transformation of $NADP^+$ to NADPH.

LL-DAP was needed for the dehydrogenase assay. Although the chiral ene approach discussed in Chapter 2 (Schemes 11 and 13)[53] can potentially be applied to make stereochemically pure LL-DAP, it was more convenient to follow the literature procedure[141-144] to resolve unprotected LL-DAP and *meso*-DAP (Scheme 36) in large quantity. This process depends on selective crystallization of the bis-*N*-Cbz-DAP (**131a, b, c**) to remove the *meso*-isomer **131a** from LL- and DD-isomers **131b, c**, followed by formation of bis-carboxyamides, removal of *N*-Cbz, and asymmetric hydrolysis by leucine aminopeptidase.

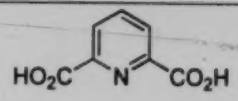
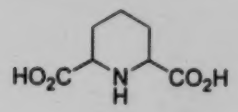
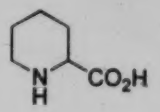
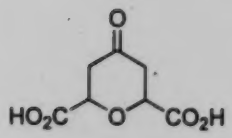
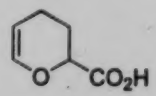
With DAP dehydrogenase and pure LL-DAP available, transition-state and substrate analogues were assayed. At neutral or slightly basic conditions (e.g. pH 7.8) the dehydrogenase normally catalyzes the reductive amination of L-THDP to *meso*-DAP with concomitant conversion of NADPH to $NADP^+$, which allows continuous spectrophotometric assay at 340 nm. In contrast, pH 10.5 is the optimum for the reverse reaction, oxidative deamination of *meso*-DAP. However, the oxidative deamination reaction can also be forced to proceed at pH 7.8 by providing the enzyme with $NADP^+$ and *meso*-DAP in the absence of added ammonium ions.

Commercial heterocycles **133 - 137** (Table 6), which have similar structural features to L-THDP were examined as substrate analogues. However, these compounds


HO2C
CO2H
NH2
NH2
1a, b, c (meso, LL, DD-DAP)
88%
Cbz-Cl
NaOH
NHCbz
NHCbz
131
selective recrystallization
131b, c (LL, DD)
131a (meso)
46%
1. Et3N, ClOCCH2CHMe2
2. NH4OH
3. H2, Pd/C
62%
H2, Pd/C
H2NOC
CONH2
132b, c (LL, DD)
1a (meso-DAP)
40%
leucine aminopeptidase
1b (LL-DAP)

Scheme 36.

Table 6. Inhibition Tests at pH 7.8 for a Forced Deamination Reaction (I)

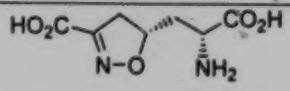
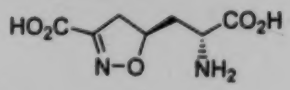
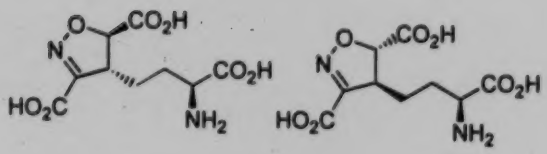
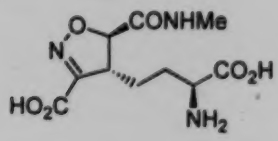
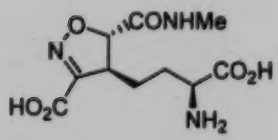
no.	structure	% inhibition (1 mM)[a]
133		33
134		37
135		37
136		50.4
137		27

[a] in buffer C containing 0.25 mM NADP, DAP dehydrogenase (480 μU) and 0.33 mM *meso*-DAP.

are only weak inhibitors of dehydrogenase at pH 7.8 against *meso*-DAP and show no inhibition of oxidative deamination at pH 10.5. This supports K. Soda and coworker's early observations[129] that DAP dehydrogenase has very tight substrate specificity. In that study,[129] none of the 30 amino acids and 10 α-keto acids they tested as mimics of natural substrates *meso*-DAP and L-THDP showed any activity against this enzyme.

Isoxazolines **104a**, **104b**, **105a**, **105b**, **106** and **107** (Table 7) which were synthesized, and tested against DAP epimerase as described above, were also examined

Table 7. Inhibition Tests at pH 7.8 for a Forced Deamination Reaction (II)

no.	structure	inhibition[a]
106		70% (240 μM)
107		38% (500 μM)
104a & 104b		17.1% (500 μM)
105a		~5% (1mM)
105b		~5% (1mM)

[a] in buffer C containing 0.25 mM NADP, DAP dehydrogenase (480 μU) and 0.33 mM *meso*-DAP.

as inhibitors of DAP dehydrogenase. However, inhibition tests at pH 7.8 for a forced oxidative deamination reaction show that **104a**, **104b**, **105a** and **105b** are relatively inactive toward DAP dehydrogenase. This may be due to steric influence caused by extra substituents on C-5' or incorrect conformation fixed by bulky isoxazoline rings which can not be accommodated by the enzyme.

Interestingly, the isoxazolines **106** and **107** with D-configuration at the amino acid site show better inhibition against DAP dehydrogenase (Table 7). At pH 7.8 for a forced oxidative deamination, (2*R*, 5*S*)-isoxazoline **106** gives 70% inhibition at 390 μM and (2*R*, 5*R*) **107** displays 38% inhibition at 500 μM. Like the previously reported (2*S*, 5*S*)-isoxazoline **99**,[23] their activities drop quite dramatically with no detectable inhibition under pH 10.5 assay conditions. Among the four isoxazoline compounds **106**, **107**, **99** and **100**, the sequence of activity toward DAP dehydrogenase is: (2*S*, 5*S*) **99** > (2*R*, 5*S*) **106** > (2*R*, 5*R*) **107** > (2*S*, 5*R*) **100**. This suggests that the stereochemistry of the isoxazoline rings and remote amino acids plays important roles for enzyme recognition. Generally DAP analogues containing isoxazoline rings with *S*-configuration (**99** and **106**) are stronger inhibitors than the corresponding *R*-analogues (**107** and **100**). The enzyme also prefers a distal L-amino acid (**99**), to its D-counterpart (**106**). However, the fact that **106** is active adds an additional layer of stereochemical complexity since the remote L-amino acid of **99** would be expected to occupy the L-amino acid binding site for *meso*-DAP in the dehydrogenase.

To address some of these problems, our second approach for mechanistic studies on DAP dehydrogenase was a collaboration with Dr. J. S. Blanchard's group. This involved x-ray crystallographic studies with this enzyme soaked with inhibitor **99** (the synthesis of this compound was summarized in Scheme 32). They have successfully solved and refined the three-dimensional structures of *C. glutamicum* DAP dehydrogenase as a binary complex with the substrate *meso*-DAP, and a ternary complex with $NADP^+$ and isoxazoline **99**, with x-ray diffraction data to 2.2 Å.[145] Their work

shows that the enzyme is a homodimer with each monomer composed of three domains: a dinucleotide binding domain for $NADP^+$ (A1 through A4, and A10 in Figure 16), a dimerization domain (B6, B10 and B11, and A5 in Figure 16) and a substrate binding domain for *meso*-DAP (A6 through A9, and B7 through B9 in Figure 16). The bound *meso*-DAP is found within a cavity formed by main chain and side chain-atoms of residues that belong to all three protein domains (Figure 17): Asp 90-His 92 and Leu 265-Pro 271 from the dinucleotide binding domain, Gly 118-Met 122, Thr 142-Ser 149 and His 244 from the dimerization domain, and Gln 150-Gly 151, Thr 169 and Arg 195 from the substrate domain. The fourth side is solvent exposed and water molecules form hydrogen bonds with two amino groups of *meso*-DAP.

Surprisingly, the isoxazoline inhibitor **99** is bound to the enzyme in a different way than expected (Figure 18). The inhibitor occupies the same site as *meso*-DAP, but it has its L-amino acid center positioned where the D-center of *meso*-DAP would normally be located, and places its sp^2-hybridized isoxazoline center where the L-center of *meso*-DAP would be. The orientation clearly shows that the isoxazoline **99** does not act as an α-planar transition-state analogue as we anticipated. This kind model of 'wrong binding' generates a non-productive complex which inhibits the enzyme in a very potent competitive fashion.

The three-dimensional structures of DAP dehydrogenase have explained the complexity of the stereochemistry of these isoxazoline compounds. A model building of (2*S*, 5*R*) **100** (Figure 19) shows that in order to maintain the maximum number of interactions of the two carboxylates with the residues of the protein, the aliphatic portion

of the isoxazoline ring of **100** is exposed to the solvent, and the polar nitrogen and oxygen atoms on the ring are forced against the protein which causes loss of hydrogen bonds with solvent water molecules. These non-covalent interactions may explain the strong binding of **99** to DAP dehydrogenase and the lack of activity of its diastereomer **100**.

In summary, DAP epimerase and dehydrogenase have been explored by the testing of a number of heterocyclic compounds as transition-state analogues and/or substrate analogues. The low activity of these isoxazolines against DAP epimerase suggests that the lack of an amino function on the isoxazoline rings may cause problems in recognition by the epimerase. DAP dehydrogenase does not recognize our L-THDP analogues, but accepts isoxazoline compounds with remote D-centers. X-ray crystallography shows isoxazoline **99** is not a transition-state inhibitor for the dehydrogenase but a non-productive enzyme binder.

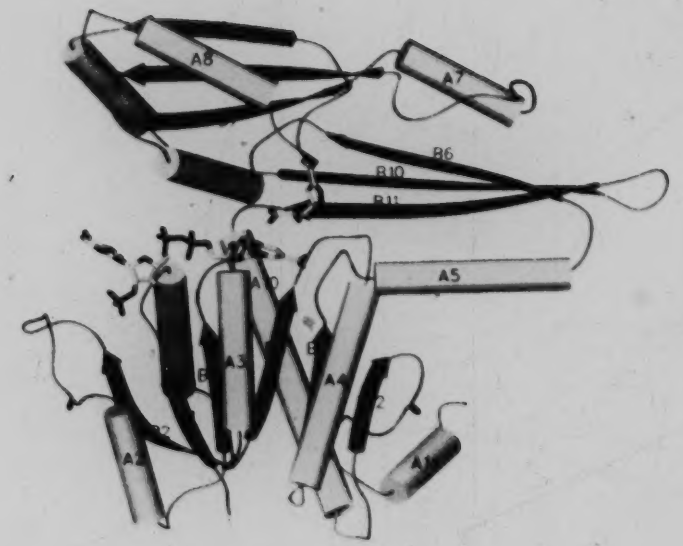


Figure 16. Ribbon Diagram of the *C. glutamicum* DAP DH Monomer

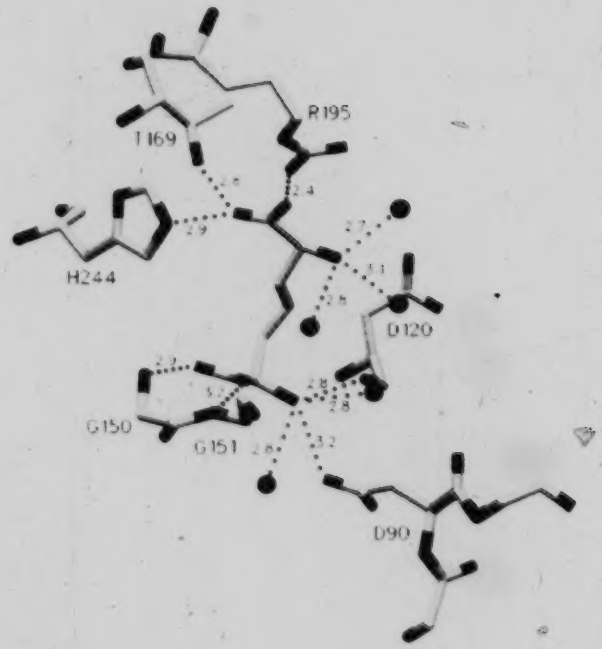


Figure 17. DAP Binding Site

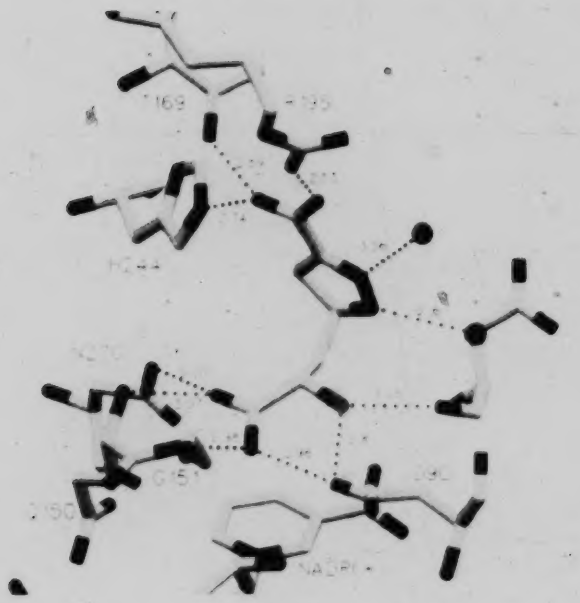


Figure 18. Isoxazoline **99** Binding Site

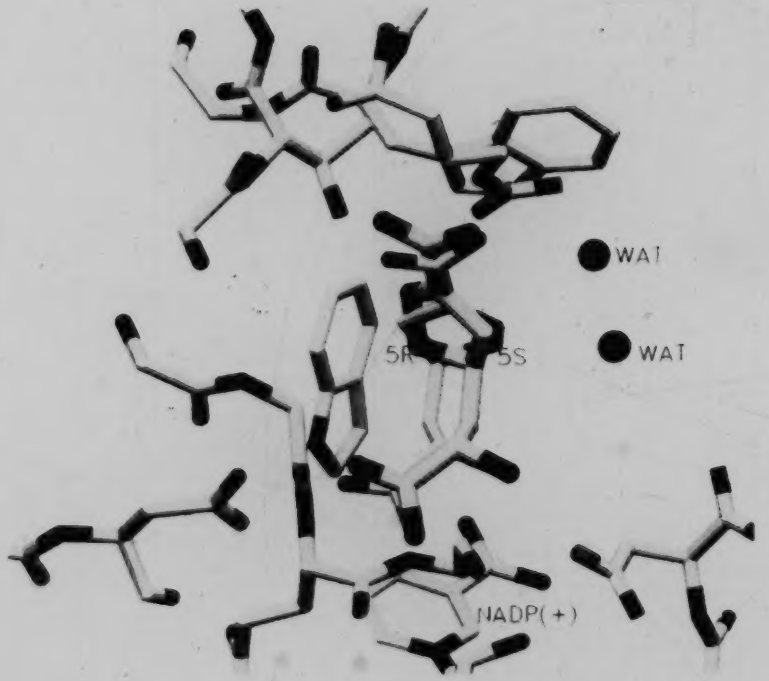


Figure 19. Overlay of **99** and **100** in the Substrate Binding Site

EXPERIMENTAL

General

All reagents employed were of ACS grade or finer, and were used without further purification unless otherwise mentioned. Diazomethane (CH_2N_2) was prepared using Diazide™.[155] *n*-Butyllithium solution was periodically titrated against methanol/phenanthroline.[147]

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware. The removal of solvent *in vacuo* refers to evaporation below 40 °C on a rotary evaporator, followed by evacuation (<0.1 mm Hg) to constant sample weight. Solvents for anhydrous reactions were dried according to Perrin *et al.*[148] THF, diethyl ether, benzene and toluene were distilled over sodium and benzophenone under an argon atmosphere. Acetonitrile, dichloromethane, DMF, DMSO, triethylamine, pyridine, hexamethyldisilazane and *N,N*-diisopropylethylamine were distilled from calcium hydride. Carbon tetrachloride and chloroform were prepared by distillation from P_2O_5. Ethanol and methanol were distilled from magnesium turnings and a catalytic amount of iodine. For highly sensitive organometallic reactions, all solvents were further degassed by at least three cycles of freeze-and-thaw in a Schlenk tube and were transferred *via* an air tight syringe. Water was obtained from a Milli-Q reagent water system (Millipore Co., Milford, MA). "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, KOH and NaOH refer to aqueous solutions.

When possible all reactions were followed by thin layer chromatography (TLC) and visualized using UV fluorescence; iodine staining; phosphomolybdic acid/ceric sulfate/sulfuric acid/water (10 g: 1.25 g: 12 mL: 238 mL) spray for general hydrocarbons; ninhydrin/*n*-BuOH/AcOH (0.32 g: 100 mL: 3 mL) spray for amino acids and amines.[149] When necessary, TLC plates were developed by heating with a hot air gun. Commercial thin layer and preparative thin layer chromatography plates used were: normal silica (Merck 60 F-254); reverse phase (Merck RP-8 or RP-18 F-254S); cation exchange resin (Polygram® Ionex-25 SA-Na). The developing solvent for amino acids on cation exchange resin TLC was either sodium citrate buffer (pH 5.0) or 0.5 N NH_4Cl. TLC of amino acids on silica used either *n*-BuOH/AcOH/H_2O (4:2:1) or *i*-PrOH/aqueous ammonia (7:3) as solvents. Flash chromatography was performed according to Still *et al.*[150] using Merck type 60, 230-240 mesh silica gel and argon pressure. The cation exchange resins used were BioRad AG 50, 50 wx 8 (H^+ form, 50-100 mesh). Microcrystalline cellulose for cellulose chromatography was purchased from Merck.

Normal phase medium pressure liquid chromatography (MPLC) used a column of Merck Kieselgel 60 H (*ca.* 55 g, 2.5 x 30 cm) and reverse phase MPLC was performed on Merck Lobar Lichroprep RP-8 columns. HPLC separations were performed on a BioRad Model 400 system equipped with a model 166 variable wavelength UV detector and an Altex 210A injector with a 100 µL sample loop. The columns used were Waters C_{18} Resolve 10 µm or Nova-Pak C_{18} 4 µm. HPLC grade solvents were purified by filtration with a Millipore filtration system under vacuum before use.

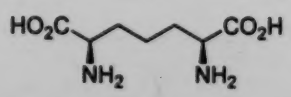
Melting points were determined on a Thomas-Hoover or Büchi immersion apparatus using open capillary tubes and were uncorrected. Optical rotations were

measured on a Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature. All specific rotations reported were measured at the sodium D line and were referenced against air. Infrared (IR) were recorded on a Nicolet 7199 or 20SX FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on Kratos AEI MS-50 (high resolution, electron impact ionization (EI)), MS-12 (chemical ionization (CI), NH_3), and MS-9 (fast atom bombardment (FAB), argon) instruments. Cleland matrix used in FAB refers to a 5:1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained on Perkin Elmer 240 or Carlo Erba 1180 elemental analyzers.

Nuclear magnetic resonance (NMR) spectra were obtained on Bruker WH-200, AM-300, WM-360, and WH-400 instruments. ^{1}H NMR chemical shifts were reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual proton resonance of solvents as the reference: $CDCl_3$ δ 7.25, CD_2Cl_2 δ 5.32, D_2O δ 4.72, CD_3OD δ 3.30 (CD_3), $(CD_3)_2SO$ δ 2.49, $(CD_3)_2CO$ δ 2.04, CD_3CN δ 1.93. ^{13}C NMR shifts were reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, CD_3OD δ 49.0, $(CD_3)_2SO$ δ 39.5, $(CD_3)_2CO$ δ 29.8 (CD_3), CD_3CN δ 117.7 (CN). Selective homonuclear decoupling, attached proton test (APT) were used for signal assignments.[151] ^{1}H NMR data were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; q, quintet; m, multiplet), number of protons, coupling constant(s) in Hertz (Hz) (resolution: 0.8 Hz), and assignment.

For the enzyme work, dialysis was performed with Spectropor tubing sizes 2 and 4 obtained from Fisher. DEAE cellulose was obtained from Whatman while the

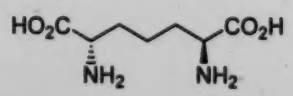
Sephadex gel was purchased from Sigma. Hydroxy apatite for chromatography was BioRad Bio-Gel HTP. Ultrafiltration was performed with an Amicon Stirred Ultrafiltration Cell, Model 8200, with a YM-10 membrane (10,000 MW cut-off), under argon pressure. Column chromatography was performed with an LKB peristaltic pump to control the flow rate on BioRad Econo-Columns with fittings. Centrifugation was performed at 4 °C with a Sorvall® RC-5B centrifuge equipped with SS-34, SM-24, GSA, and GS-3 motors. Spectrophotometric assays were performed on a Hewlett Packard (HP) 8452A diode array spectrophotometer controlled by a HP Vectra ES computer loaded with HP89530A MS DOS-UV/VIS operating software, and equipped with a water-jacketed single cell holder.



***meso*-Diaminopimelic Acid (1a) from 42.** The procedure of Lane-Bell for deprotection was adapted.[52] To a solution of **42** (14 mg, 0.033 mmol) in methanol (15 mL) and glacial AcOH (1 mL) was added 10% Pd/C (10 mg). The suspension was stirred under 1 atm of H_2 for 14 h and then filtered through cotton wool. The filtrate was evaporated *in vacuo* to give a powder, to which was added a mixture of CF_3CO_2H (15 mL) and CH_2Cl_2 (15 mL). The resulting solution was stirred at room temperature for 15 min and then evaporated *in vacuo*. The residue was purified by ion exchange chromatography (AG50W x 8, H^+ form, 5 mL), eluting with H_2O and 2% to 10% aqueous NH_4OH to give pure **1a** (3.9 mg, 64%) with chromatographic and spectral properties in

agreement with **1a** (*meso*-DAP) obtained by classical resolution-separation procedures[141-144] from **131a** as described below.

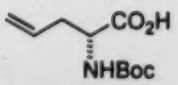
(2*R*, 6*S*)-2,6-Diaminopimelic Acid (1a) from 131a. The procedure of Arnold was adapted.[141] A solution of **131a** (6.2 g, 13.5 mmol) in acetic acid (30 mL) and H_2O (10 mL) with 10% Pd/C was stirred under 1 atm of H_2 for 12 h. The catalyst was removed by passing through a bed of Celite and the filtrate was concentrated *in vacuo*. The residue was redissolved in H_2O (10 mL) and evaporation repeated. The solid was dissolved in a minimum amount of hot H_2O, and the hot solution was filtered. The pH was adjusted to 6.0 with 1 M LiOH and **1a** crystallized by addition of hot EtOH and slow cooling to 0 °C. The crystals were filtered and dried to give *meso*-DAP (**1a**) (1.6 g, 62%): $[\alpha]_D$ = 0.00 ° (*c* 2.0, 1 M HCl); IR (KBr) 3300-2400 (br), 1631, 1599, 1510, 1503 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.50-1.80 (m, 2 H, $CH_2CH_2CH_2$), 1.90-2.16 (m, 4 H, $CH_2CH_2CH_2$), 4.14 (t, 2 H, *J* = 6.3 Hz, 2 x CH); FAB MS *m/z* (Cleland) 191 ($[M+1]^+$).



(2*S*, 6*S*)-2,6-Diaminopimelic Acid (1b). The procedure of Arnold was adapted.[141] A mixture of **132b** and **132c** (2.1 g, 6.82 mmol) was incubated for 72 h at ambient temperature with leucine aminopeptidase (3000 units, from hog kidney, 7 mL of 200 units/mg in 2.9 M $(NH_4)_2SO_4$) in 0.01 M manganous acetate at pH 8.0. The enzyme was removed by ultrafiltration using an Amicon apparatus (PM-10 membrane) and the filtrate was applied on a column of BioRad AG 50 Wx 8. The column was eluted successively with H_2O and 0.5 M NH_3. The early H_2O fractions containing **1b** were pooled and evaporated *in vacuo* to give a white solid which was further purified by a

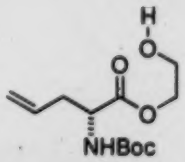
BioRad AG50 Wx 8 (H^+ form, 100 mL of bed volume), eluting with H_2O (200 mL), 0.5 M NH_3 (200 mL). The fractions of **1b** were combined and evaporated *in vacuo*. The solid was recrystallized from H_2O/MeOH to give **1b** (257 mg, 40%): $[\alpha]_D$ = +45.1 ° (*c* 1.37 HCl); IR and ^1H NMR spectra were essentially as described for **1a** as above. Incubation of **1b** (10 mM) with DAP dehydrogenase showed no measurable activity (free of *meso*-DAP).

Analysis of Diastereomeric Excess of DAP Isomers 1a, 1b and 1c. In a typical procedure,[87] to a saturated aqueous $NaHCO_3$ (2 mL) solution of **1a** (2.8 mg, 0.015 mmol) was added (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (11 mg, 0.033 mmol). After stirring at room temperature for 1 h, the aqueous solution was washed with EtOAc (1 mL), acidified to pH 2 by adding 2 M HCl and extracted with EtOAc (5 x 10 mL). The extracts were dried and evaporated *in vacuo* to give a residue which was purified by HPLC (the Mosher amides of different DAP isomers do not separate under these conditions): (BioRad Model 400 system; solid phase - C_{18} Resolve 10 μm, two 8 mm x 10 cm columns in tandem; solvent A: 0.1% CF_3CO_2H in H_2O; solvent B: CH_3CN; Method: gradient 5%-70% in 35 min, at 1 mL/min, detection at 254 nM; retention time: 27.71 min). ^1H NMR ($CDCl_3$, 500 MHz) analysis of the methoxy region allowed discrimination between all DAP isomers and provided the isomeric ratios of DAP synthesized by the various procedures.



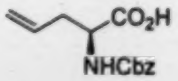
***N*-(*tert*-Butoxycarbonyl)-D-allylglycine (3).** The procedure of Cox et al.[19] was adapted. To a solution of D-allylglycine **138** (0.61 g, 5.3 mmol) in saturated aqueous

$NaHCO_3$ (60 mL) and 1,4-dioxane (10 mL) was added Boc_2O (1.4 g, 6.4 mmol). After stirring for 5 h, the solution was washed with EtOAc (20 mL), acidified to pH 1 with 2 M HCl and extracted with EtOAc (3 x 30 mL). The organic extracts were dried and evaporated to give **3** as an oil (0.68 g, 61%): R_f 0.67 (70% EtOAc in hexane); $[\alpha]_D$ = - 13.33 ° (*c* 1.08 $CHCl_3$); IR ($CHCl_3$ cast) 3340-3000, 1736, 1712, 1620, 1592 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H, $C(CH_3)_3$); 2.42-2.62 (m, 2 H, CH_2CHNH), 4.38 (dd, 1 H, *J* = 7.6, 4.8 Hz, *CH*NH), 5.02-5.21 (m, 2 H, CH_2=CH), 5.65-5.80 (m, 1 H, CH_2=*CH*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.27 ($C(CH_3)_3$), 36.40 (CH_2CHNH), 52.76 (*C*HNH), 80.30 ($C(CH_3)_3$), 119.36 (CH_2=CH), 132.14 (CH_2=*C*H), 155.53 ($NHCO_2$), 176.61 (*C*OOH); CI MS *m/z* (NH_3) 233 (48, $[M+18]^+$), 216 (48, $[M+1]^+$); Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 56.36; H, 8.17; N, 6.42.

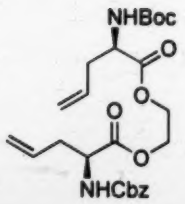


***N*-(*tert*-Butoxycarbonyl)-D-allylglycine, 2-Hydroxyethyl Ester (4).** Condensation of **3** and ethylene glycol analogous to preparation of **15** below gave **4** in 82% yield: R_f 0.28 (40% EtOAc in hexane); IR ($CHCl_3$ cast) 3428, 3215, 1770, 1729, 1620, 1587 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 9 H, $C(CH_3)_3$), 2.41-2.59 (m, 2 H, CH_2CHNH), 3.79 (t, 2 H, *J* = 4.7 Hz, CH_2OH), 4.20-4.28 (t, 2 H, *J* = 4.7 Hz, CH_2CH_2OH), 4.28-4.34 (t, 1 H, *J* = 6.6 Hz, *CH*NH), 5.08-5.21 (m, 2 H, CH_2=CH), 5.63-5.74 (m, 1 H, CH_2=*CH*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.26 ($C(CH_3)_3$), 36.40 (CH_2CHNH), 53.13 (*C*HNH), 60.60 (CH_2OH), 66.73 (CH_2CH_2OH), 80.26 ($C(CH_3)_3$),

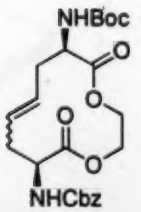
119.21 (CH_2=CH), 132.37 (CH_2=CH), 155.64 ($NHCO_2$), 172.26 ($COOH$); CI MS *m/z* (NH_3) 277 (62, $[M+18]^+$); Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.81; H, 8.36; N, 5.14.



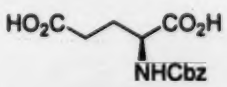
***N*-(Benzyloxycarbonyl)-L-allylglycine (5).** The procedure of Cox et al.[19] was adapted. To a solution of L-allylglycine **45** (1.52 g, 13.2 mmol) in saturated K_2CO_3 (20 mL) stirred at 0 °C was added benzyl chloroformate (3.34 g, 19.8 mmol) dropwise. The pH was maintained at 9 by the addition of 2 M NaOH solution as necessary. After 20 min, the basic solution was washed with CH_2Cl_2 (2 x 30 mL) and the aqueous layer was acidified to pH 2 with 1 M HCl solution which was extracted with ether (4 x 50 mL). The combined ethereal layers were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to afford **5** as an oil (2.66 g, 81%): $[\alpha]_D$ = +15.9 (*c* 0.88 $CHCl_3$); IR ($CHCl_3$ cast) 3320, 1720, 1521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.52-2.70 (m, 2 H, CH_2CHNH), 4.55 (ddd, 1 H, *J* = 6.1, 6.6, 8.0 Hz, CHNH), 5.22 (d, 1 H, *J* = 8.0 Hz, N*H*), 5.68-5.78 (m, 4 H, CH_2=CH and CH_2Ph), 5.68-5.78 (m, 1 H, CH_2=CH), 7.31-7.47 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 36.39 (CH_2CHNH), 53.19 (CHNH), 67.29 (CH_2Ph), 119.70 (CH_2=CH), 128.17, 128.29, 128.58 (aromatic *C*H's), 131.86 (CH_2=CH), 136.08 (quaternary aromatic C), 156.13 ($NHCO_2$), 176.32 ($COOH$); exact mass 249.1003 (249.1001 calcd for $C_{13}H_{15}NO_4$); Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.89; H, 6.21; N, 5.45.



2-((2*R*)-2-((*tert*-Butoxycarbonyl)amino)-4-pentenoyloxy)ethyl *N*-(Benzyloxy-carbonyl)-L-allylglycinate (6). Condensation of **4** and *N*-Cbz-L-allylglycine (**5**) analogous to preparation of **15** below gave **6** in 83% yield: R_f 0.60 (30% EtOAc in hexane); $[\alpha]_D$ = -3 ° (*c* 0.5 $CHCl_3$); IR ($CHCl_3$ cast) 3347, 1747, 1716, 1642, 1518 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.41 (s, 9 H, $C(CH_3)_3$), 2.39-2.62 (m, 4 H, 2 x CH_2CHNH), 4.35 (s, 4 H, OCH_2CH_2O), 4.42 (q, 1H, *J* = 7.5 Hz, CHNH), 4.97-5.08 (dd, 1 H, *J* = 5.8, 7.7 Hz, CHNH), 5.08-5.14 (m, 4 H, 2 x CH_2=CH), 5.20 (s, 2 H, CH_2Ph), 5.35 (d, 1 H, *J* = 7.5 Hz, NH), 5.59-5.72 (m, 2 H, 2 x CH_2=CH), 7.27-7.38 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.29 ($C(CH_3)_3$), 36.54 (CH_2CHNH), 37.12 (CH_2CHNH),52.86 (CHNH), 53.27 (CHNH), 62.58, 62.83 (OCH_2CH_2O), 67.04 (CH_2Ph), 80.00 ($C(CH_3)_3$) 119.27, 119.53 (CH_2=CH), 128.11, 128.21, 128.53 (aromatic CH's), 131.91, 132.18 (CH_2=CH), 136.23 (quaternary aromatic C), 155.10, 155.76 (2 x $NHCO_2$), 171.48, 171.84 (2 x COOH); CI MS *m/z* (NH_3) 492 (27, $[M+1]^+$); Anal. Calcd for $C_{25}H_{34}N_2O_8$: C, 61.21; H, 6.99; N, 5.71. Found: C, 61.39; H, 7.06; N, 5.47.

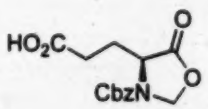


(6*R*, 11*S*)-11-(*N*-(Benzyloxycarbonyl)amino)-6-(*N*-(*tert*-butoxycarbonyl)-amino)-5,12-dioxo-1,4-dioxacyclododec-8-ene (8). The procedure of Zuercher et al. for olefin metathesis was adapted.[55] All reagents and solvents were dried and degassed. A round-bottomed flask charged with catalyst bis(tricyclohexylphosphine)-benzylidine ruthenium dichloride (7) (17 mg, 0.021 mmol) (Strem Chemicals, Newburyport, MA) was evacuated and filled with argon 3 times before the addition of **6** (50.8 mg, 0.104 mmol) in degassed CH_2Cl_2 (15 mL) by an air-tight syringe. The resulting orange solution was stirred under argon for 1.5 h and then stirred for 2 h exposed to the air. The solution color changed from orange to dark black. Removal of the solvent *in vacuo* gave an oily residue which was purified by flash chromatography (25% EtOAc in hexane) to afford **8** as an equal mixture of double bond isomers (17 mg, 35%): R_f 0.09 (20% EtOAc in hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (s, 9 H, C(C*H*$_3$)$_3$), 2.27-2.48 (m, 2 H, C*H*$_2$CHNH), 2.48-2.68 (m, 2 H, C*H*$_2$CHNH), 4.22-4.59 (m, 6 H, OC*H*$_2$C*H*$_2$O, 2 x C*H*NH), 5.12 (s, 2 H, C*H*$_2$Ph), 5.42-5.50 (m, 2 H, C*H*=C*H*), 7.30-7.42 (m, 5 H, Ph); MS CI *m/z* (NH_3) 480 (33, $[M+18]^+$), 463 (41, $[M+1]^+$).



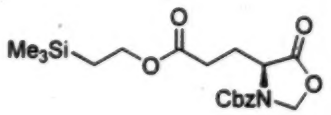
***N*-(Benzyloxycarbonyl)-L-glutamic Acid (13).** The procedures of Greenstein et al.[152] and Cheng[153] were adapted to prepare this known compound. To a stirred solution

of L-glutamic acid (30 g, 204 mmol) in saturated $NaHCO_3$ solution (900 mL) cooled in an ice-water bath was added benzyl chloroformate (54.0 g, 317 mmol) over 15 min. The ice-water bath was then allowed to warm up to room temperature over 3 h. After stirring for another 10 h, the aqueous solution was washed with CH_2Cl_2 (300 mL), acidified to pH 1 by conc. HCl, extracted by ether (3 x 500 mL). The combined ethereal layers were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give **13** (48.9 g, 85 %): IR (KBr) 3305, 1710, 1690, 1578 cm^{-1}; ^1H NMR (NaOD, 400 MHz) δ 2.15-2.17 (m, 2 H, CH_2CHNH), 2.43-2.46 (t, 2 H, J = 7.2 Hz, HO_2CCH_2), 4.42 (t, 1 H, J = 7.8 Hz, $CHNH$), 5.13 (s, 2 H, CH_2Ph), 7.28-7.34 (m, 5 H, Ph); FAB MS *m/z* (Cleland) 282 (15 , $[M+1]^+$); Anal. Calcd for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.21; H, 5.42; N, 4.99.



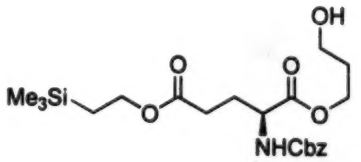
(4*S*)-3-(3-*N*-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl)propanoic Acid (14). The procedure of Itoh et al. to prepare this known compound has been adapted.[154] *N*-CBz-L-glutamic acid (**13**) (7.75 g, 27.5 mmol), paraformaldehyde (1.5 g) and *p*-TsOH (0.31 g, 1.8 mmol) were dissolved in freshly distilled benzene (130 mL). After stirring the solution briefly, the flask was fitted with a Dean Stark trap and a condenser. The solution was brought to reflux in an oil bath for 5 h and the reaction mixture was washed with water (2 x 20 mL) and saturated aqueous $NaHCO_3$ (2 x 25 mL). The combined $NaHCO_3$ aqueous solution was acidified to pH 1 with 2 M HCl and then extracted with EtOAc (3 x 125 mL). The combined organic layers were dried and evaporated *in vacuo* to give a residue which was purified by flash chromatography (40% EtOAc in hexane) to

afford **14** as an oil (4.83 g, 60%): R_f 0.19 (40% EtOAc in hexane); IR ($CHCl_3$ cast) 3200, 1801, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.10-2.43 (m, 2 H, CH_2CHN), 2.48 (t, 2 H, J = 7.2 Hz, HO_2CCH_2), 4.41 (t, 1 H, J = 6.0 Hz, CHN), 5.16 (s, 2 H, CH_2Ph), 5.25-5.50 (m, 2 H, OCH_2N), 7.35 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 25.71 (CH_2CHN), 29.07 (HO_2CCH_2), 53.89 (CHN), 68.23 (CH_2Ph), 77.85 (OCH_2N), 128.35, 128.71, 135.15 (aromatic CH's), 153.07 (quaternary aromatic C), 171.61 (CO_2CH_2), 177.69 ($COOH$); CI MS *m/z* (NH_3) 311 (100, $[M+18]^+$), 294 (14, $[M+1]^+$); Anal. Calcd for $C_{14}H_{15}NO_6$: C, 57.34; H, 5.16; N, 4.78. Found: C, 57.11; H, 5.22; N, 4.65.



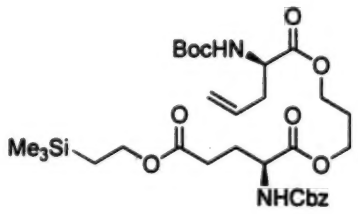
2-(Trimethylsilyl)ethyl (4*S*)-3-(3-*N*-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl)-propanoate (15). The procedure of Diago-Meseguer et al. for alcohol coupling was adapted.[155] To a stirred solution of compound **14** (233 mg, 0.789 mmol), 2-(trimethylsilyl)ethanol (113 μL, 0.789 mmol) and *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (201 mg, 0.789 mmol) in CH_2Cl_2 (30 mL) was added Et_3N (220 μL, 1.58 mmol). After 10 h, the solvent was evaporated *in vacuo* to give a residue which was purified by flash chromatography (30% EtOAc in hexane) to afford **15** as an oil (158 mg, 51%): R_f 0.92 (60% EtOAc in hexane); IR ($CHCl_3$ cast) 1802, 1724, 1453 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ -0.03 (s, 9 H, $Si(CH_3)_3$), 0.91 (t, 2 H, J = 8.6 Hz, CH_2Si), 2.01-2.38 (m, 4 H, CH_2CH_2CHN), 4.07 (t, 2 H, J = 8.6 Hz, CH_2CH_2Si), 4.29 (t, 1 H, J = 5.8 Hz, CHN), 5.16 (s, 2 H, CH_2Ph), 5.21-5.40 (m, 2 H, OCH_2N), 7.19-7.28 (m, 5 H, Ph); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ -1.92 ($Si(CH_3)_3$), 16.88 (CH_2Si), 25.55

(CH_2CHN), 29.08 (CH_2CH_2CHN), 53.63 (CHN), 62.39 (CH_2CH_2Si), 67.44 (CH_2Ph), 77.42 (OCH_2N), 127.82, 128.08, 128.23 (aromatic CH's), 135.15 (quaternary aromatic C), 152.60 (NCO_2), 171.37 ($CH_2O_2CCH_2$), 171.70 (CH_2CO_2CH); CI MS *m/z* (NH_3) 411 (35, $[M+18]^+$); Anal. Calcd for $C_{19}H_{27}NO_6Si$: C, 57.99; H, 6.92; N, 3.56. Found: C, 58.10; H, 7.21; N, 3.26.



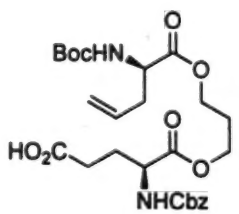
***N*-(Benzyloxycarbonyl)-L-glutamic Acid, α-(3-Hydroxy)propyl γ-(2-Trimethylsilyl)ethyl Ester (16).** The procedure of Hanessian and Sahoo to make vinylglycine was adapted.[64] Propane-1,3-diol (34 μL, 0.47 mmol) in THF (10 mL) was treated with 2.5 M *n*-BuLi in hexane (188 μL, 0.47 mmol). After stirring at room temperature for 10 min, this solution was transferred to a solution of **15** (154 mg, 0.392 mmol) in THF (20 mL) *via* cannula. The resulting mixture was stirred for 12 h and the solvent was removed *in vacuo*. The residue was re-dissolved in EtOAc (40 mL), washed with water (10 mL), dried and evaporated *in vacuo* to give an oil. Purification by flash chromatography (60% EtOAc in hexane) afforded **16** as a yellow oil (80 mg, 47%): R_f 0.43 (60% EtOAc in hexane); $[\alpha]_D$ = +6.7 ° (*c* 7.2 $CHCl_3$); IR (nujol) 3079-2853, 1642, 1611, 1586, 1559, 1512 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.01 (s, 9 H, $Si(CH_3)_3$), 0.97 (t, 2 H, *J* = 8.4 Hz, CH_2Si), 1.85 (t, 2 H, *J* = 5.9 Hz, CH_2CH_2OH), 2.05-2.17 (m, 2 H, CH_2CHN), 2.38 (t, 2 H, *J* = 6.1 Hz, CH_2CH_2CHN), 3.67 (t, 2 H, *J* = 5.9 Hz, CH_2OH), 4.16 (t, 2 H, *J* = 8.4 Hz, CH_2CH_2Si), 4.27 (t, 2 H, *J* = 5.9 Hz, $CH_2CH_2CH_2OH$), 4.37 (m, 1

H, C*H*N), 5.15 (s, 2 H, CH_2Ph), 5.42 (d, 1 H, *J* = 7.7 Hz, N*H*), 7.26-7.33 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -1.58 (Si(*C*H_3)$_3$), 17.24 (*C*H_2Si), 27.35 (*C*H_2CH$_2$OH), 30.21 (*C*H_2CHN), 31.34 (*C*H_2CH$_2$CHN), 53.47 (*C*HN), 58.76, 62.51, 63.02 (*C*H_2CH$_2$*C*H$_2$OH, *C*H_2CH$_2$Si), 67.03 (*C*H_2Ph), 128.04, 128.15, 128.49 (aromatic *C*H's), 136.12 (quaternary aromatic C), 155.96 (NH*C*O$_2$), 172.14 (*C*O$_2$CH$_2$), 172.89 (*C*OOH); FAB MS *m/z* (Cleland) 440 (2.6, $[M+1]^+$); Anal. Calcd for $C_{21}H_{33}NO_7Si$: C, 57.38; H, 7.57; N, 3.19. Found: C, 57.29; H, 7.62; N, 3.15.



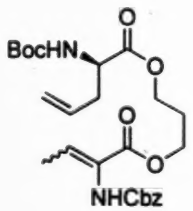
***N*-(Benzyloxycarbonyl)-L-glutamic Acid, α-3-((2*R*)-2-(*N*-(*tert*-Butoxy-carbonyl)amino)-4-pentenoyloxy)propyl γ-2-(Trimethylsilyl)ethyl Ester (17).** The procedure of Boden and Keck for esterification was adapted.[156] A flask was charged with dicyclohexycarbodiimide (DCC) (60 mg, 0.32 mmol), 4-(dimethylamino)pyridine (DMAP) (59 mg, 0.48 mmol) and DMAP.HCl (51 mg, 0.32 mmol) in CH_2Cl_2 (20 mL). The resulting solution was brought to reflux and a solution of **16** (70 mg, 0.16 mmol) and (*R*)-(*N*-*tert*-butoxycarbonyl)allylglycine (**3**) (34 mg, 0.16 mmol) in CH_2Cl_2 (10 mL) was added. After 16 h, the solution was cooled to room temperature and washed with 1 M HCl (10 mL) and saturated aqueous $NaHCO_3$ solution. Hexane (10 mL) was added and the resulting suspension was filtered, the filtrate being evaporated *in vacuo* to give an oily residue which was purified by flash chromatography (30% EtOAc in hexane) to afford **17** (90 mg, 88%): R_f 0.28 (20% EtOAc in hexane); $[\alpha]_D$ = +0.33 (*c* 0.3 $CHCl_3$); IR ($CHCl_3$

cast) 3353, 1724, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.05 (s, 9 H, $Si(CH_3)_3$), 0.95 (t, 2 H J = 8.5 Hz, CH_2Si), 1.42 (s, 9 H, $C(CH_3)_3$), 1.98-2.40 (m, 4 H, $OCH_2CH_2CH_2O$, $CH_2CHNHCbz$), 2.41-2.60 (m, 4 H, $CH_2=CHCH_2$, CH_2CH_2CHNH), 4.15 (t, 2 H, J = 8.5 Hz, CH_2CH_2Si), 4.18-4.26 (m, 4 H, $OCH_2CH_2CH_2O$), 4.30-4.42 (m, 2 H, 2 x CHN) 5.07 (d, 1 H, J = 7.8 Hz, NH), 5.10 (s, 2 H, OCH_2Ph), 5.13-5.17 (m, 2 H, $CH_2=CH$), 5.48 (d, 1 H, J = 7.8 Hz, NH), 5.48-5.73 (m, 1 H, $CH_2=CH$), 7.27-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ -1.52 ($Si(CH_3)_3$), 17.30 (CH_2Si), 27.47, 27.88 ($OCH_2CH_2CH_2O$, CH_2CHNH), 28.30 ($C(CH_3)_3$), 30.36 ($CH_2CH_2CHNHCbz$), 36.69 ($CH_2=CHCH_2$), 52.91, 53.48 (2 x $CHNH$), 61.61 (CH_2CH_2Si), 62.05, 63.01 ($OCH_2CH_2CH_2O$), 67.07 (CH_2Ph), 79.96 ($C(CH_3)_3$), 119.18 ($CH_2=CH$), 128.11, 128.20, 128.54 (aromatic CH's), 132.27 ($CH_2=CH$), 136.20 (quaternary aromatic C), 155.23 ($NHCO_2$), 155.98 ($NHCO_2$), 171.80, 172.00, 172.77 (3 x CO_2CH_2); FAB MS *m/z* (Cleland) 637 (2, $[M+1]^+$); Anal. Calcd for $C_{31}H_{48}N_2O_{10}Si$: C, 58.47; H, 7.60; N, 4.40. Found: C, 58.78; H, 7.54; N, 4.21.



***N*-(Benzyloxycarbonyl)-L-glutamic Acid, α-3-((2*R*)-2-(*N*-(*tert*-Butoxy-carbonyl)amino)-4-pentenoyloxy)propyl Ester (18).** The procedure of Campbell and Hart for deprotection was adapted.[88] To a solution of **17** (83 mg, 0.13 mmol) in THF (10 mL) was added a 1 M solution of TBAF in THF (400 μL, 0.4 mmol). The resulting red solution was stirred for 45 min and then evaporated *in vacuo*. The residue was dissolved

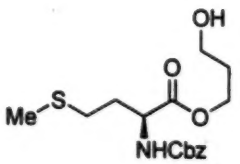
in Et_2O (45 mL) and the ethereal solution was washed with 1 M HCl (20 mL), dried and evaporated *in vacuo* to give a gray oil **18** (70 mg, quantitative): R_f 0.62 (EtOAc); IR ($CHCl_3$ cast) 3200-2800, 1773, 1760, 1596 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) 1.42 (s, 9 H, $C(CH_3)_3$), 1.96-2.22 (m, 4 H, $OCH_2CH_2CH_2O$, $CH_2CHNHCbz$), 2.40-2.48 (m, 4 H, CH_2=$CHCH_2$, CH_2COOH), 4.16-4.30 (m, 4 H, $OCH_2CH_2CH_2O$), 4.30-4.45 (m, 2 H, 2 x CHNH), 5.07-5.18 (m, 5 H, CH_2=CH, CH_2Ph, NH), 5.56 (d, 1 H, J = 7.8 Hz, NH), 5.61-5.78 (m, 1 H, CH_2=CH), 7.26-7.40 (m, 5 H, Ph); CI MS *m/z* (NH_3) 537 (42, $[M+1]^+$).



Lead Tetraacetate Oxidation of 18 to Generate 19. The procedure of Hanessian and Sahoo to make vinylglycine was adapted.[64] To a solution of **18** (70 mg, 0.13 mmol) in benzene (20 mL) was added cupric acetate monohydrate (8 mg, 0.0373 mmol) and the suspension was stirred under argon for 1 h. Freshly prepared lead tetraacetate (132 mg, 0.299 mmol) was added and the resulting suspension was stirred for 1 h at room temperature then 15 h at reflux. The suspension was then cooled and diluted with EtOAc (20 mL). The mixture was washed with water (3 x 20 mL), brine (15 mL), dried and evaporated *in vacuo*. The resulting syrup was purified by flash chromatography (30% EtOAc in hexane) to give **19** (28 mg, 43%): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9 H, $C(CH_3)_3$), 1.80 (d, 3 H, J = 7.2 Hz, CH_3CH=C), 1.94-2.11 (m, 2 H, $OCH_2CH_2CH_2O$),

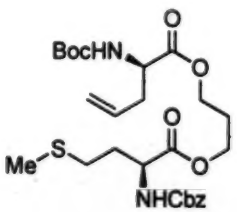
2.41-2.59 (m, 2 H, CH_2CHNH), 4.12-4.27 (m, 5 H, O$CH_2$$CH_2$$CH_2$O, CHNH), 5.03 (d, 1 H, J = 7.8 Hz, NH), 5.09-5.18 (m, 5 H, CH_2=CH, CH_2Ph, NH), 5.65-5.76 (m, 1 H, CH_2=CH), 6.74 (q, 1 H, J = 7.2 Hz, CH_3CH=C), 7.26-7.40 (m, 5 H, Ph).

Conversion of 22 to 19. The procedure of Carrasco et al. was adapted.[158] Compound **22** (60 mg, 0.108 mmol) in a round-bottomed flask was rotated in a Kugelrohr apparatus (195-200 °C, 0.1-0.3 mmHg pressure) for 1 h to give a black residue which was purified by flash chromatography (30% EtOAc in hexane) to afford **19** as a single product (30 mg, 57%). The spectral and physical properties were in accord with those of **19** prepared from **18**.

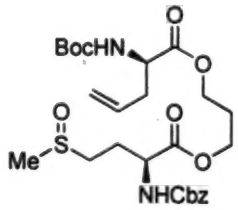


***N*-(Benzyloxycarbonyl)-L-methionine, 3-Hydroxypropyl Ester (20).** The procedures of Carrasco et al.[158] and Greenstein and Winitz[152] were adapted. To a solution of L-methionine (1.0 g, 6.7 mmol) in saturated aqueous $NaHCO_3$ (20 mL) solution was added benzyl chloroformate (1.11 mL, 7.4 mmol) dropwise over 2 min. After stirring at room temperature for 2 h, the solution was washed with EtOAc (20 mL), acidified to pH 2 with 2 M HCl and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried and evaporated *in vacuo* to give a yellow oil (1.2 g, 63%). A solution of this oil (721 mg) with propane-1,3-diol (968 mg, 12.74 mmol) in CH_2Cl_2 (10 mL) was transferred to a solution of DCC (524 mg, 2.55 mmol), DMAP (310 mg, 2.55 mmol) and DMAP.HCl (405 mg, 2.55 mmol) in CH_2Cl_2 (20 mL) at reflux. After 6h at reflux, the solution was cooled to room temperature and washed with 1 M HCl (2 x 20 mL). The

organic layer was dried and evaporated *in vacuo* to give a residue which was purified by flash chromatography (40% EtOAc in hexane) to afford **20** (685 mg, 79%): R_f 0.14 (40% EtOAc in hexane); IR ($CHCl_3$ cast) 3400-2900, 1721, 1523 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.58-1.98 (m, 4 H, CH_2CHN, $OCH_2CH_2CH_2O$), 2.09 (s, 3 H, SCH_3), 2.55 (t, 2 H, J = 7.8 Hz, CH_3SCH_2), 3.69 (t, 2 H, J = 5.9 Hz, CH_2OH), 4.34 (m, 2 H, $CH_2(CH_2)_2OH$), 4.45 (m, 1 H, CHNH), 5.12 (s, 2 H, CH_2Ph), 5.41 (d, 1 H, J = 7.8 Hz, NH), 7.22-7.26 (m, 5 H, Ph); CI MS *m/z* (NH_3) 359 (25, $[M+18]^+$); Anal. Calcd for $C_{16}H_{23}NO_5S$: C, 56.29; H, 6.79; N, 4.10. Found: C, 56.64; H, 6.97; N, 3.98.

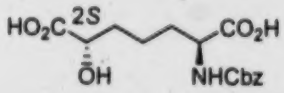


***N*-(Benzyloxycarbonyl)-L-methionine, 3-((2*R*)-2-(*N*-(*tert*-Butoxycarbonyl)-amino)-4-pentenoyloxy)propyl Ester (21).** The analogous method was used as for the preparation of **17** to give compound **21** as an oil in 84% yield: R_f 0.63 (40% EtOAc in hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 9 H, $C(CH_3)_3$), 1.86-2.41 (m, 7 H, CH_3S, $OCH_2CH_2CH_2O$, $CH_2CHNHCbz$), 2.37-2.60 (m, 4 H, $CH_2=CHCH_2$, CH_3SCH_2), 4.04-4.26 (m, 4 H, $OCH_2CH_2CH_2O$), 4.35 (m, 1 H, CHNH), 4.48 (m, 1 H, CHNH), 5.00-5.18 (m, 4 H, CH_2Ph, $CH_2=CH$), 5.42 (d, 1 H, J = 7.8 Hz, NHBoc), 5.54 (d, 1 H, J = 7.8 Hz, NH), 5.60-5.71 (m, 1 H, $CH_2=CH$), 7.23-7.26 (m, 5 H, Ph); CI MS *m/z* (NH_3) 556 (34, $[M+18]^+$), 539 (20, $[M+1]^+$).

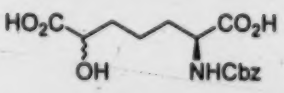


***N*-(Benzyloxycarbonyl)-L-methionine Sulfoxide, 3-((2*R*)-2-(*N*-(*tert*-Butoxy-carbonyl)amino)-4-pentenoyloxy)propyl Ester (22).** The procedure of Liu was adapted.[159] To a cooled solution (0 °C) of **21** (150 mg, 0.28 mmol) in methanol (10 mL) was added a solution of $NaIO_4$ (63 mg, 0.29 mmol) in water (10 mL) dropwise over a period of 2 h through an addition funnel. The mixture was then stirred at 4 °C for 12 h. The product was filtered through a bed of Celite and the filtrate was extracted with chloroform (6 x 50 mL). The combined organic layers were washed with water (20 mL), brine (20 mL), dried and evaporated *in vacuo*. The residue was purified by flash chromatography (EtOAc) to give an oil **22** (127 mg, 82%): R_f 0.12 (EtOAc); IR ($CHCl_3$ cast) 3340, 1770, 1720, 1580 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.93-2.50 (m, 6 H, 2 x CH_2CHNH, $OCH_2CH_2CH_2O$), 2.54 (s, 3 H, CH_3SO), 2.77 (t, 2 H, J = 7.6 Hz, CH_3SOCH_2), 4.10-4.26 (m, 4 H, $OCH_2CH_2CH_2O$), 4.33 (m, 1 H, CHNH), 4.46 (m, 1 H, CHNH), 5.08 (s, 2 H, CH_2Ph), 5.11-5.19 (m, 2 H, CH_2=CH), 5.60-5.78 (m, 1 H, CH_2=CH), 5.83 (d, 1 H, J = 8.0 Hz, NH), 5.89 (d, 1 H, J = 7.8 Hz, NH), 7.37-7.41 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.87 ($OCH_2CH_2CH_2O$), 28.33 ($C(CH_3)_3$), 32.27 ($CH_3SOCH_2CH_2$), 36.63 (CH_2=$CHCH_2$), 38.54 (CH_3SO), 50.07 (CH_3SOCH_2) 52.95(CHNH), 53.21 (CHNH), 61.52, 62.32 ($OCH_2CH_2CH_2O$), 67.20 (CH_2Ph), 80.01 ($C(CH_3)_3$), 119.20 (CH_2=CH), 128.19, 128.29, 128.57 (aromatic CH's), 132.31 (CH_2=CH), 136.14 (quaternary aromatic C), 155.30, 156.13 (2 x NHCO), 171.19, 172.06

($CO_2(CH_2)_3O_2C$); CI MS *m/z* (NH_3) 572 (23, $[M+18]^+$), 555 (17, $[M+1]^+$); Anal. Calcd for $C_{26}H_{38}N_2O_9S$: C, 56.30; H, 6.91; N, 5.05. Found: C, 56.71; H, 6.77; N, 5.17.



Dimethyl (2*S*, 6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydroxyheptane-1,7-dioate ((2*S*)-23) from 33. The procedure of Back and coworkers for nickel boride reduction was adapted.[162,163] Compound **33** (150 mg, 0.327 mmol) and $NiCl_2 \cdot 6H_2O$ (0.53 g, 2.28 mmol) were dissolved in 9 mL of methanol and 3 mL of THF in a flask cooled in an ice bath with stirring. $NaBH_4$ (254 mg, 6.8 mmol) was added in portions over 15 min. The immediate formation of a black precipitate was observed, and the mixture was stirred for an additional 15 min. The precipitate was filtered through a bed of Celite and the filtrate was evaporated to dryness *in vacuo*. Purification of the residue by flash chromatography (20% EtOAc in hexane) gave (2*S*)-**23** as an oil (97 mg, 85%) with spectral and chromatographic properties in agreement with those for **23** from **48**. Conversion to DAP and NMR analysis of the corresponding Mosher amides showed an isomeric ratio 2S:2*R* of 94:6. $[\alpha]_D = +37.23$ (*c* 0.5 $CHCl_3$).

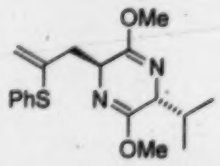


Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydroxy-heptane-1,7-dioate (23) from 48. The procedure of Cox et al. to make this compound was adapted.[19] Compound **48** (1.92 g, 8.77 mmol) was dissolved in CH_2Cl_2 (20 mL) and benzyl chloroformate (1.56 mL, 11 mmol) was added. Pyridine (0.883 mL, 11 mmol) was added *via* syringe over 5 min, and the solution was stirred for 90 min before the addition of 1 M

HCl (20 mL). The mixture was extracted into CH_2Cl_2 (4 x 40 mL), and the combined extracts were dried and evaporated *in vacuo*. Purification by flash chromatography (70% EtOAc in hexane) gave **23** as a yellow oil (2.91 g, 94%): R_f 0.79 (EtOAc); IR (CH_2Cl_2 cast) 3356, 1790-1760, 1735, 1527 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.40-1.58 (m, 2 H, $CH_2CH_2CH_2$),1.58-1.95 (m, 4 H, $CH_2CH_2CH_2$) 3.70 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 4.08-4.20 (t, 1 H, J = 4.2 Hz, CHOH), 4.31-4.41 (m, 1 H, CHNH), 5.17 (s, 2 H, CH_2Ph), 5.20-5.31 (d, 1 H, J = 7.2 Hz, NH), 7.29-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.73 ($CH_2CH_2CH_2$), 32.36, 33.70 (2 x CH_2CH), 52.43 (OCH_3), 52.61 (OCH_3), 53.68 (CHNH), 67.07 (CH_2Ph), 70.13 (CHOH), 128.15, 128.23, 128.57 (aromatic CH's), 136.24 (quaternary aromatic C), 156.20 ($NHCO_2$), 172.81 (CO_2Me), 175.43 (CO_2Me); exact mass 353.1468 (7.3, M^+) (353.1474 calcd for $C_{17}H_{23}NO_7$); Anal. Calcd for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.42; H, 6.65; N, 3.87.

Dimethyl (2*S*,6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydroxy-heptane-1,7-dioate ((2*S*)-23) from 50. The procedure of Noyori and coworkers for asymmetric reduction was adapted.[89] All the reagents and solvents were pre-dried and degassed. To a dry Schlenk tube charged with dimethyl (6*S*)-6-(*N*-benzyloxycarbonyl)amino-2-oxo-heptane-1,7-dioate (**50**) (180 mg, 0.513 mmol) and methanol (5 mL) was added the *in situ* prepared (*S*)-Binap-Ru(II) complex (2 mg) under a stream of argon. The resulting yellow orange solution was further degassed by two freeze-thaw cycles and transferred to a dry, argon filled autoclave which was evacuated by vacuum and refilled with hydrogen five times. Finally, the system was kept at 4 atm hydrogen, 100 °C for 4h. After cooling the autoclave to room temperature and allowing excess hydrogen to bleed off, the solution

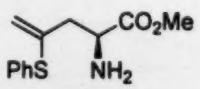
was evaporated *in vacuo*. The residue was separated by flash chromatography (40% EtOAc in hexane) to give (2*S*)-**23** as an oil (163 mg, 90%) with spectral and chromatographic properties in agreement with those for **23** prepared above from **48**. Conversion to DAP and NMR analysis of the corresponding Mosher amides (see Scheme 13) showed an isomeric ratio 2*S*:2*R* of 79:21 (58% d.e.).



(3*R*, 6*S*)-3-Isopropyl-2,5-dimethoxy-6-(2-phenylthio-2-propenyl)-3,6-dihydro-pyrazine (28). The alkylating agent 2-(phenylthio)-3-chloropropene was prepared by a modification of the literature method[157] and was used without purification. Thus, allene (0.61 g, 15.2 mmol) was bubbled into a solution of dry THF (25 mL) at -30 °C at a constant flow rate. Sulfenyl chloride (1.871 g, 12.9 mmol) in THF (5 mL) was then added dropwise (10 min). The resulting solution was stirred at -30 °C to -20 °C for about 30 min to give a solution of 2-(phenylthio)-3-chloropropene in THF.

A cooled solution (-78 °C) of (*R*)-Schöllkopf bis-lactim ether **27** (2.32 mL, 12.9 mmol) in THF (20 mL) under argon was treated with a 2.5 M solution of *n*-BuLi in hexane (5.2 mL, 12.9 mmol). After stirring for an additional 10 min at -78 °C, the THF solution of 2-(phenylthio)-3-chloropropene was added *via* cannula over 15 min and the resulting orange-black solution was stirred at -60 °C for 4 h. After another 12 h, while the temperature was gradually raised to room temperature, the solvent was removed *in vacuo* and the black residue was partitioned between Et_2O (20 mL) and water (20 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the combined organic

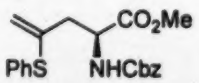
extracts were dried and evaporated. The dark oily residue was purified by flash chromatography (5% EtOAc in hexane) to give **28** as an oil (3.86 g, 90%): R_f 0.22 (10% EtOAc in hexane); IR ($CHCl_3$ cast) 1779, 1721, 1635, 1578 cm^{-1}; ^1H NMR (CD_2Cl_2, 300 MHz) δ 0.68 (d, 3 H, J = 6.9 Hz, $CHCH_3$), 1.07 (d, 3 H, J = 6.9 Hz, $CHCH_3$), 2.20-2.32 (m, 1 H, $CH(CH_3)_2$), 2.52-2.65 (m, 2 H, CH_2=$CHCH_2$), 3.18 (s, 3 H, OCH_3), 3.21 (s, 3 H, OCH_3), 3.92-3.95 (m, 1 H, N=CCH_2), 4.19-4.29 (m, 1 H, N=CCH_2), 4.92 (br s, 1 H, HHC=CSPh), 5.20 (br s, 1 H, HHC=CSPh), 7.20-7.47 (m, 5 H, Ph); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ 16.66, 19.36, 31.99, 41.37, 61.17, 61.27, 116.46, 128.07, 129.55, 132.89, 133.22, 134.17, 142.11, 163.08, 164.13; CI MS *m/z* (NH_3) 333 (100, $[M+1]^+$); Anal. Calcd for $C_{18}H_{24}N_2O_2S$: C, 65.03; H, 7.28; N, 8.43; S, 9.64. Found: C, 65.04; H, 7.24; N, 8.41; S, 9.97.



Methyl (2*S*)-4-(Phenylthio)allylglycinate (29). The procedures of Schöllkopf et al.[160] and Klinge[161] were adapted. A solution of **28** (3.0 g, 9.3 mmol) in 0.4 N CF_3CO_2H (70 mL, 28 mmol) and THF (10 mL) was stirred at room temperature for 4 h. The THF was removed *in vacuo*, and the aqueous residue was washed with EtOAc (15 mL) before it was concentrated to a volume of 15-20 mL. The pH was adjusted to 8-9 by adding saturated aqueous Na_2CO_3 solution. The mixture was extracted with EtOAc (3 x 25 mL) and the combined organic extracts were washed with brine (15 mL). The organic solution was dried and evaporated *in vacuo*. The resulting oil was bulb-to-bulb distilled and (*R*)-valine methyl ester was the forerun separated from the residue that yielded relatively

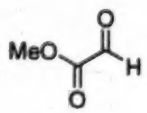
unstable **29** (1.73 g, 79%) as an oil: R_f 0.19 (EtOAc); $[\alpha]_D$ = +21.31 ° (*c* 1.60 $CHCl_3$); IR ($CHCl_3$ cast) 3379, 3316, 1739, 1607 cm^{-1}; ^1H NMR (CD_2Cl_2, 300 MHz) δ 2.40-2.70 (m, 2 H, C*H*$_2$CHNH$_2$), 3.65 (s, 3 H, OC*H*$_3$), 3.77 (t, 1 H, *J* = 6.1 Hz, C*H*NH$_2$), 5.02 (br s, 1 H, C*H*H=CSPh (*H cis*- to SPh)), 5.26 (br s, 1 H, C*H*H=CSPh (*H trans*- to SPh)), 7.30-7.49 (m, 5 H, Ph); ^{13}C NMR (CD_2Cl_2, 75.5 MHz) δ 42.45 (*C*H$_2$CHNH$_2$), 52.21 (OC*H*$_3$), 54.58 (*C*HNH$_2$), 110.79 (*C*H$_2$=CSPh), 128.46, 129.69, 133.12 (aromatic *C*H's), 133.51 (quaternary aromatic C), 142.05 (CH$_2$=*C*SPh), 175.53 (*C*O$_2$Me); exact mass 237.0823 (11, M^+) (237.0823 calcd for $C_{12}H_{15}NO_2S$).

To determine the enantiomeric excess, a stirred solution of **29** (11.1 mg, 0.047 mmol) and (1*S*)-camphanic acid chloride (15.5 mg, 0.071 mmol) in CH_2Cl_2 (10 mL) was treated with Et_3N (10 μL, 0.071 mmol).[52] After 1h, the solution was washed with 1 M HCl (10 mL), dried and evaporated *in vacuo* to give a residue. ^1H NMR ($CDCl_3$, 360 MHz) analysis showed an e.e. ≥ 98%.

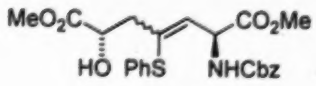


Methyl (2*S*)-*N*-(Benzyloxycarbonyl)-4-(phenylthio)allylglycinate (30). The procedure of Cox et al. was adapted.[19] To a solution of **29** (1.72 g, 7.3 mmol) and benzyl chloroformate (Cbz-Cl) (1.25 mL, 8.76 mmol) in CH_2Cl_2 (40 mL) was added freshly distilled pyridine (0.71 mL, 8.76 mmol) over 5 min, and the mixture was stirred at room temperature for 4 h. The mixture was then washed with 1 M HCl (10 mL) and brine (10 mL). The organic layer was dried and evaporated *in vacuo* to afford an oil that was purified by flash chromatography (30% EtOAc in hexane) to obtain **30** as an oil (2.69 g,

98%): R_f 0.48 (30% EtOAc in hexane); $[\alpha]_D$ = +16.22 ° (*c* 1.0 $CHCl_3$); IR ($CHCl_3$ cast) 3345, 1721, 1583 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.58-2.81 (m, 2 H, C*H*$_2$CHNH), 3.72 (s, 3 H, OC*H*$_3$), 4.62 (dt, 1 H, *J* = 6.4, 7.8 Hz, C*H*NH), 4.95 (s, 1 H, C*H*H=CSPh (*H cis-* to SPh)), 5.08-5.17 (m, 3 H, C*H*$_2$Ph, C*H*H=CSPh (*H trans-* to SPh)), 5.48 (d, 1 H, *J* = 7.8 Hz, N*H*), 7.25-7.45 (m, 10 H, 2 x Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 38.69(*C*H$_2$CHNH), 52.32 (*C*HNH), 52.96 (O*C*H$_3$), 66.95 (*C*H$_2$Ph), 116.80 (*C*H$_2$=CSPh), 128.03, 128.11, 128.18, 128.47, 129.26, 132.22 (aromatic *C*H's), 133.24 (CH$_2$=*C*SPh), 136.27, 140.33 (quaternary aromatic Cs), 155.61 (NH*C*O$_2$), 171.75 (*C*O$_2$Me); CI MS *m/z* (NH_3) 372 (80, $[M+1]^+$); Anal. Calcd for $C_{20}H_{21}NO_4S$: C, 64.67; H, 5.70; N, 3.77; S, 8.63. Found: C, 64.60; H, 5.49; N, 3.67; S, 8.90.

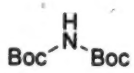


Methyl Glyoxylate (32). The procedure of Kelly et al. to make this known compound was adapted.[166] Periodic acid (45.6 g, 200 mmol) was added in portions over 1 h to a stirred solution of dimethyl DL-tartrate (35.6 g, 200 mmol) in ether (500 mL) under argon with cooling (0 °C). The cloudy suspension was stirred for 2 h until a clear solution and a white precipitate formed. The mixture was filtered and the filtrate was dried for 12 h over activated molecular sieves (4Å, 30 g). Concentration *in vacuo* and distillation under vacuum (1.5 mm Hg) into a cooled receiving flask at -78 °C afforded **32** as a viscous pale yellow oil: bp 30 °C at 1.5 mmHg; IR (CH_2Cl_2 cast) 2960, 1756, 1441 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.41 (s, 1 H, C*H*O), 3.95 (s, 3 H, OC*H*$_3$); exact mass 88.0165 (88.0160 calcd for $C_3H_4O_3$).

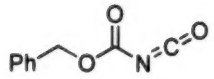


Dimethyl (2*S*, 6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydroxy-4-(phenylthio)-4-heptene-1,7-dioate (33). The procedure of Johannsen and Jörgensen for ene reaction was adapted.[81] To a dry flask under argon containing copper trifluoromethanesulfonate (60 mg, 0.084 mmol) and (*R*)-2,2'-isopropylidenebis(4-phenyl-2-oxazoline) (**31**) (61 mg, 0.084 mmol) was added dry CH_2Cl_2 (3 mL) under argon, and the mixture was stirred for 2 h. The resulting green homogenous solution was then treated with methyl glyoxylate (**32**) (70 mg, 0.8 mmol) and **30** (315 mg, 0.84 mmol) in CH_2Cl_2 (1 mL) at room temperature. After 12 h, the solution was diluted by adding CH_2Cl_2 (20 mL), washed with brine (20 mL), dried and evaporated *in vacuo* to give a yellow oil which was purified by flash chromatography (20% EtOAc in hexane) to give **33** as an oil (154 mg, 42%): R_f 0.22 (40% EtOAc in hexane); $[\alpha]_D$ = +82.91 (*c* 2.0 $CHCl_3$); IR ($CHCl_3$ cast) 3366, 1790, 1743, 1514 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.80-2.88 (m, 2 H, C*H*$_2$CH=CH), 3.78 (s, 6 H, 2 x OC*H*$_3$), 4.09 (d, 1 H, *J* = 6.8 Hz, O*H*), 4.59 (m, 1 H, C*H*OH), 5.11 (s, 2 H, C*H*$_2$Ph), 5.21 (dd, 1 H, *J* = 9.0, 6.2 Hz, C*H*NH), 5.42 (d, 1 H, *J* = 9.0 Hz, PhSC=C*H*), 5.76 (d, 1 H, *J* = 6.2 Hz, N*H*), 7.20-7.51 (m, 10 H, 2 x Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.63 (*C*H$_2$CSPH=CH), 52.40 (O*C*H$_3$), 52.94 (O*C*H$_3$), 53.10 (*C*HNH), 67.31 (*C*H$_2$Ph), 69.22 (*C*HOH), 127.55 (PhSC=*C*H), 128.07, 128.20, 128.30, 128.58, 129.37, 132.54 (aromatic *C*H's), 135.98 (quaternary aromatic C), 138.64 (S*C*=CH), 155.62 (NH*C*O$_2$), 170.42 (*C*O$_2$Me), 173.89 (*C*O$_2$Me); FAB MS *m/z* (Cleland)

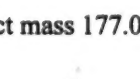
481 (26, $[M+Na]^+$), 460 (1, $[M+H]^+$); Anal. Calcd for $C_{23}H_{25}NO_7S$: C, 60.12; H, 5.48; N, 3.05. Found: C, 59.60; H, 5.23; N, 2.98.



Di-*tert*-butyl Iminodicarbonate (34). The procedure of Degerbeck et al. was adapted.[85] Well-dried, finely powdered NH_4Cl (268 mg, 5.0 mmol) was suspended in a solution of Boc_2O (5.49 g, 25 mmol) in dry CH_3CN (15 mL) under argon at ambient temperature. The resulting slurry was then treated with DMAP (1.22 g, 10.0 mmol) dissolved in dry CH_3CN (10 mL) with rapid stirring over a period of 1 h. The vigorous evolution of gas gradually subsidized after a few hours and the stirring was continued for another 23 h under argon. The resulting clear yellow solution was treated with vigorous agitation with 2-diethylaminoethylamine (DEAEA) (2.32 g, 20 mmol) over a period of 1 h, and the reaction mixture was stirred for a further 12 h. Removal of the solvent *in vacuo* left a brownish thick oil which was partitioned between ether (200 mL) and 1 M $KHSO_4$ (50 mL). The ethereal extract was washed with 1 M $KHSO_4$ (25 mL), 1 M $KHCO_3$ (25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give **34** as a powder (1.48 g, 85%): R_f 0.68 (66% toluene in acetonitrile); IR ($CHCl_3$ cast) 3318, 2945, 1764 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.42 (s, 18 H, 2 x $C(CH_3)_3$); CI MS *m/z* (NH_3) 235 (39, $[M+18]^+$), 218 (5, $[M+1]^+$); Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.52; H, 9.03; N, 6.57.

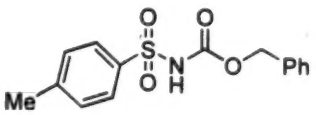


(Benzyloxycarbonyl)isocyanate (35). The procedure of Grehn et al. was adapted.[86] To a suspension of dry, finely grounded benzyl carbamate (5.2 g, 34 mmol) in dry CH_2Cl_2 (50 mL) under argon was added dropwise a solution of oxalyl chloride (4.36 mL, 50 mmol) in CH_2Cl_2 at 0 °C over a period of 1 h. The clear solution became turbid gradually. Stirring was continued for 4 h at ambient temperature and for 15 h under reflux. The mixture was then concentrated to about 2/3 of its original volume and the precipitate was filtered and washed with cold, dry CH_2Cl_2 (3 x 10 mL). The combined pale yellow filtrate was evaporated *in vacuo* to dryness, and the residual yellowish turbid oil was distilled at reduced pressure (0.2 Torr). After a forerun, consisting largely of oxalyl chloride and benzyl chloride (40 - 45 °C/0.15 - 0.20 Torr), **35** was collected as a colorless liquid (75 - 80 °C/0.15 - 0.20 Torr) (1.4 g, 24%): bp 76-79 °C (0.17 Torr); IR (KBr) 3286, 1786, 1741, 1709, 1506 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.22 (s, 2 H, C*H*$_2$Ph), 7.37-7.48 (m, 5 H, Ph); exact mass 177.0425 (177.0426 Calcd for $C_9H_7NO_3$).

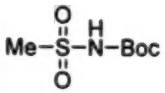


Dibenzyl Iminodicarbonate (36). The procedure of Grehn et al. was followed.[86] To a vigorously stirred solution of benzyl alcohol (0.97 g, 9.0 mmol) in dry CH_2Cl_2 (30 mL) under argon and cooled in ice, was added a solution of **35** (1.4 g, 7.91 mmol) in dry CH_2Cl_2 (10 mL) dropwise over a period of 30 min. After the addition, the colorless solution was stirred under argon at 0 °C for 1 h and at ambient temperature for 15 h. The solution was evaporated *in vacuo* and the residue was treated with cold petroleum ether

(10 mL). The resultant solution was kept at 4 °C for 12 h. The white crystalline solid was collected by filtration to afford **36** (2.14 g, 90%): IR (KBr) 3201, 1813, 1772, 1708, 1561 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.19 (s, 4 H, 2 x C*H*$_2$), 7.05 (s, 1 H, N*H*), 7.26-7.38 (m, 10 H, 2 x Ph); CI MS *m/z* (NH_3) 303 (100, $[M+18]^+$), 286 (29, $[M+1]^+$); exact mass 285.0999 (285.1001 Calcd for $C_{16}H_{15}NO_4$); Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.38; H, 5.62; N, 4.88.

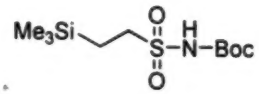


Benzyl *N*-(*p*-Tolylsulfonyl)carbamate (37). The procedure of Grehn et al. was adapted.[86] Toluene-*p*-sulfonyl isocyanate (1.52 mL, 10.0 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and the solution was added dropwise to a rapidly stirred, ice-cooled suspension of benzyl alcohol (1.03 g, 9.5 mmol) in dry CH_2Cl_2 (20 mL) during 30 min. The resulting solution was stirred for 1 h at 0 °C and for 12 h at ambient temperature. After evaporation of the solvent *in vacuo*, the solid residue was purified by recrystallization from CH_2Cl_2/Et_2O to afford **37** as white crystals (2.4 g, 78%): mp 103-104 °C (lit[86] mp: 103-105 °C); IR (KBr) 3279, 1735, 1497 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 2.48 (s, 3 H, C*H*$_3$Ph), 5.11 (s, 2 H, C*H*$_2$Ph), 7.25-8.0 (m, 9 H, 2 x Ph); CI MS *m/z* (NH_3) 323 (9, $[M+18]^+$); Anal. Calcd for $C_{15}H_{15}NSO_4$: C, 59.00; H, 4.95; N, 4.59; S, 10.50. Found: C, 58.63; H, 4.94; N, 4.63; S, 10.49.

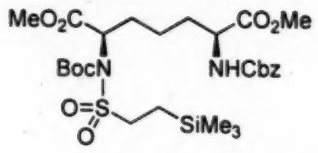


***tert*-Butyl *N*-(Methylsulfonyl)carbamate (38).** The procedure of Campbell and Hart to make this known compound was adapted.[88] To a solution of methanesulfonamide (8.0 g, 84 mmol) in THF (200 mL) at -55 °C was added 2.5 M *n*-BuLi (33.7 mL, 84 mmol) in hexane over a period of 10 min. The resulting white suspension was cooled to -78 °C with stirring for 30 min, and then KH (35% in oil) (15 g, 132 mmol) was added. This was followed by the addition of TMEDA (23 mL, 153 mmol). The mixture was then slowly warmed to room temperature over a period of 1 h. This was cooled to -78 °C, *tert*-butyl azidoformate (15.1 g, 105 mmol) was added over a period of 10 min, and the mixture was allowed to warm to ambient temperature over 2 h. It was then heated at 40-45 °C for 17 h. *tert*-Butyl alcohol (15 mL) was added to quench the excess KH and water (5 mL) was added after 20 min at 5 °C. The resultant orange emulsion was mixed with water (100 mL), the aqueous phase was separated. This was acidified to pH 2-3 using conc. HCl at 0 °C. This aqueous phase was then extracted with CH_2Cl_2 (4 x 250 mL). The combined organic extracts were washed with 3 M HCl (100 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give a light yellow solid. The solid was diluted with ether-CH_2Cl_2-MeOH (68:8:1, 77 mL) and hexane (40 mL) was added immediately. The resulting slurry was refrigerated for 2 h and the white solid was collected. The filtrate was evaporated and the residue was crystallized again to yield a second portion of white solid. This was combined with first portion to give **38** (11.5 g, 70%): mp 119-120 °C (lit[88] mp 119-120 °C; IR (KBr) 3409, 3284, 1713, 1353 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.50 (s, 9 H, $C(CH_3)_3$), 3.25 (s, 3 H, CH_3SO_2), 7.07 (s, 1 H,

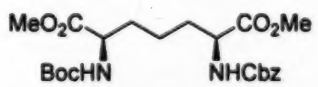
N*H*); CI MS *m/z* (NH_3) 213 (100, $[M+18]^+$); Anal. Calcd for $C_6H_{13}NSO_4$: C, 36.91; H, 6.72; N, 7.18. Found: C, 36.83; H, 6.82; N, 7.18.



***tert*-Butyl *N*-((2-(Trimethylsilyl)ethyl)sulfonyl)carbamate (39).** The procedure of Campbell and Hart to make this known compound was adapted.[88] To a solution of diisopropylamine (11.5 mL, 82.2 mmol) in THF (75 mL) at -78 °C was added a 2.5 M solution of *n*-BuLi in hexane (32 mL, 80 mmol) over a period of 5 min. The solution was stirred for another 15 min and then a solution of **38** (7.8 g, 40.0 mmol) in THF (75 mL) was added over 15 min. The mixture was stirred for 10 min and then transferred *via* a cannula to a solution of (iodomethyl)trimethylsilane (6.4 mL, 43.1 mmol) in THF (45 mL) at -78 °C over a period of 15 min. The solution was stirred for 2 h and then allowed to warm to room temperature over another 2 h. The mixture was poured into 380 mL of ice-water containing 2 M aqueous HCl (100 mL). The aqueous solution was extracted with CH_2Cl_2 (4 x 300 mL). The combined extracts were washed with saturated aqueous sodium bisulfite (150 mL), brine (120 mL), dried and concentrated *in vacuo* to give a yellow solid, which was recrystallized from 9:1 hexane/ether to yield white crystals of **39** (8.20 g, 73%): mp 80-82 °C (lit[88] mp 80-81 °C) ; IR (KBr) 3243, 1742, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 9 H, $Si(CH_3)_3$), 1.03 (t, 2 H, J = 7.2 Hz, CH_2Si), 1.50 (s, 9 H, $C(CH_3)_3$), 3.29 (t, 2 H, J = 7.2 Hz, CH_2SO_2); CI MS *m/z* (NH_3) 299 (100, $[M+18]^+$); Anal. Calcd for $C_{10}H_{23}NSSiO_4$: C, 42.69; H, 8.25; N, 4.98. Found: C, 42.66; H, 8.27; N, 4.80.

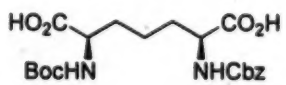


Dimethyl (2*R*, 6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(*N*-(*tert*-butoxy-carbonyl)-*N*-(2-trimethylsilylethylsulfonyl)amino)heptane-1,7-dioate (40). The procedure of Campbell and Hart for Mitsunobu reaction was adapted.[88] To a solution of Ph_3P (115 mg, 0.442 mmol), carbamate **39** (116 mg, 0.414 mmol) and (2*S*)-**23** (96 mg, 0.27 mmol) in 20 mL of THF at 0 °C was added dropwise diethyl azodicarboxylate (65 μL, 0.414 mmol) over a 5 min period. The solution was stirred for 5 h at room temperature, concentrated *in vacuo* and purified by flash chromatography (25% EtOAc in hexane) to give **40** as an oil (132 mg, 79%): R_f 0.32 (30% EtOAc in hexane); IR ($CHCl_3$ cast) 3376, 1729, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.06 (s, 9 H, Si(CH_3)$_3$), 1.02-2.22 (m, 17 H, C(CH_3)$_3$, $CH_2CH_2CH_2$, CH_2Si), 1.47 (s, 9 H, C(CH_3)$_3$), 3.40-3.60 (t, 2 H, *J* = 7.1 Hz, CH_2CH_2Si), 3.71 (s, 6 H, 2 x OCH_3), 4.30-4.40 (m, 1 H, C*H*NH), 4.68-4.75 (m, 1 H, C*H*N), 5.09 (s, 2 H, CH_2Ph), 5.30-5.37 (d, 1 H, *J* = 7.4 Hz, N*H*), 7.26-7.38 (m, 5 H, Ph); CI MS *m/z* (NH_3) 634 (19, $[M+18]^+$), 617 (1, $[M+1]^+$); Anal. Calcd for $C_{27}H_{44}N_2O_{10}SSi$: C, 52.58; H, 7.19; N, 4.54. Found: C, 52.36; H, 7.02; N, 4.68.



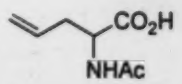
Dimethyl (2*R*, 6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(*N*-(*tert*-butoxy-carbonyl)amino)-heptane-1,7-dioate (41). To a solution of **40** (110 mg, 0.176 mmol) in 10 mL of THF was added a 1 M solution of *n*-Bu_4NF (TBAF) in THF (537 μL, 0.54 mmol). The resulting solution was stirred for 15 min and then diluted with 45 mL of

Et_2O. The organic layer was washed with water (4 x 20 mL) followed by 15 mL of saturated aqueous $NaHCO_3$, dried and concentrated *in vacuo*. The residue was purified by flash chromatography (50% EtOAc in hexane) to give **41** (78 mg, quantitative) as an oil: R_f 0.55 (50% EtOAc in hexane); $[\alpha]_D$ = +5.45 (*c* 0.5 $CHCl_3$); IR ($CHCl_3$ cast) 3348, 1741, 1716, 1521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.70-2.15 (m, 15 H, $C(CH_3)_3$, $CH_2CH_2CH_2$), 1.41 (s, 9 H, $C(CH_3)_3$), 3.70 (s, 3 H, OCH_3), 3.72 (s, 3 H, OCH_3), 4.22-4.42 (m, 2 H, 2 x CHNH), 5.09 (s, 2 H, CH_2Ph), 5.35 (m, 2 H, 2 x NH), 7.27-7.38 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.17 ($CH_2CH_2CH_2$), 28.28 ($C(CH_3)_3$), 31.90 (CH_2CHNH), 32.31 (CH_2CHNH), 52.27 (CHNH), 53.55 (CHNH), 57.06 (OCH_3) 58.01 (OCH_3), 67.51 (CH_2Ph), 79.99 ($C(CH_3)_3$), 127.75, 128.16, 128.51 (aromatic CH's), 136.21 (quaternary aromatic C), 155.10 ($NHCO_2$), 172.79 (CO_2Me), 173.03 (CO_2Me); CI MS *m/z* (NH_3) 470 (51, $[M+18]^+$), 453 (17, $[M+1]^+$); Anal. Calcd for $C_{22}H_{32}N_2O_8$: C, 58.40; H, 7.13; N, 6.19. Found C, 58.00; H, 7.15; N, 6.13.

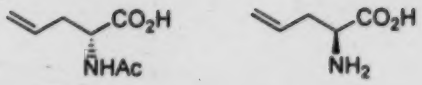


(2*R*, 6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(*N*-(*tert*-butoxycarbonyl)amino)-heptane-1,7-dioic Acid (42). The procedure of Cox et al. for ester hydrolysis was adapted.[19] A mixture of **41** (70 mg, 0.153 mmol) and 10% aqueous NaOH (340 μL, 0.82 mmol) was stirred vigorously for 4 h, then diluted with water (2 mL) and CH_3CN (3 mL). After an additional 14 h of stirring, the mixture was diluted with water (4 mL), washed with EtOAc (4 mL), acidified to pH 1 with 2 M HCl and extracted with EtOAc (3 x 20 mL). The combined extracts were dried and evaporated *in vacuo* to give **42** (60 mg,

94%): IR ($CHCl_3$ cast) 3348, 1747, 1716, 1528 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.15-1.57 (m, 11 H, $C(CH_3)_3$, $CH_2CH_2CH_2$), 1.41 (s, 9 H, $C(CH_3)_3$), 1.62-1.87 (m, 4 H, $CH_2CH_2CH_2$), 4.21-4.44 (m, 4 H, 2 x $CHCO_2H$, 2 x NH), 5.11 (s, 2 H, CH_2Ph), 7.28-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.17 ($CH_2CH_2CH_2$) 28.29 ($C(CH_3)_3$), 31.90 (CH_2CHNH), 32.31 (CH_2NH), 52.85 (CHNH), 53.55 (CHNH), 57.06 (CH_2Ph), 79.99 ($C(CH_3)_3$), 127.76, 128.17, 128.51 (aromatic CH's), 136.22 (quaternary aromatic C), 153.50 ($NHCO_2$), 153.92 ($NHCO_2$), 172.79 (CO_2H), 173.04 (CO_2H); CI MS *m/z* (NH_3) 442 (92, $[M+18]^+$), 425 (30, $[M+1]^+$).

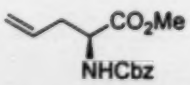


(*RS*)-*N*-Acetylallylglycine (43). The procedure of Black and Wright to make this known compound was adapted.[164] To a solution of DL-allylglycine (25 g, 217 mmol) in 2 M NaOH (120 mL) at 0 °C was added dropwise acetic anhydride (70 mL, 741 mmol). The pH was maintained at 9 by the addition of 2 M NaOH as necessary. The solution was stirred for another 30 min, then acidified with conc. HCl to pH 2. The acidic aqueous solution was extracted with EtOAc (4 x 300 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give **43** (24.1 g, 74%): R_f 0.80 (90% EtOAc in hexane); IR (EtOAc cast) 3326, 1728, 1642 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.90 (s, 3 H, CH_3CO), 2.37-2.55 (m, 2 H, CH_2CHNH), 4.32 (dt, 1 H, *J* = 7.8, 6.3 Hz, CHNH), 5.05-5.12 (m, 2 H, CH_2=CH), 5.63-5.72 (m, 1 H, CH_2=CH); exact mass 157.0736 (157.0739 calcd for $C_7H_{11}NO_3$).



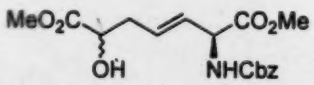
***N*-Acetyl-D-allylglycine (44) and L-Allylglycine (45).** The procedure of Black and Wright for enzyme resolution was adapted.[164] Compound **43** (24.1 g, 153 mmol) was dissolved in water (1.4 L) and the pH was adjusted to 7.8 using a saturated solution of LiOH. Porcine kidney acylase I (350 mg) was added and the solution was stirred with the temperature maintained at 35-38 °C for 16 h. Then Norit A (5 g) was added and the black mixture was filtered through a bed of Celite to give a clear solution, which was concentrated to a volume of 1 L. The pH was adjusted to 6.5 by the addition of 1 M HCl. This solution was applied to the H^+ form of BioRad AG-50 cation exchange resin (600 mL bed volume) and the aqueous washings were collected until the eluent was neutral. The column was then eluted with 1 M aqueous NH_3 solution and the eluent was collected until amino acid was fully eluted (by ninhydrin test). The amino acid fractions were combined and evaporated *in vacuo* to afford a white powder which was further purified by recrystalization from H_2O/MeOH to give **45** as white crystals (4.42 g, 50%): mp 157-159 °C (lit[164] mp 158-159 °C); $[\alpha]_D$ = -36.69 ° (*c* 4 H_2O) (lit[164] $[\alpha]_D$ = -37.1 ° (*c* 4 H_2O)); IR (KBr) 3210, 1720, 1642 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.55-2.74 (m, 2 H, CH_2CHNH_2), 3.81 (dd, 1 H, *J* = 7.0, 5.2 Hz, $CHNH$), 5.22-5.36 (m, 2 H, CH_2=CH), 5.70-5.86 (m, 1 H, CH_2=CH); ^{13}C NMR (D_2O, 75.5 Hz) δ 34.86 (CH_2CHNH_2), 54.01 ($CHNH$), 120.55 (CH_2=CH), 131.40 (CH_2=CH), 174.30 ($COOH$); exact mass 115.0632 (115.0633 calcd for $C_5H_9NO_2$); Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.18; H, 7.80; N, 12.08.

The above aqueous neutral solution was evaporated *in vacuo* to yield pale yellow residue which was recrystallized from H_2O/MeOH to afford a white crystal **44** (7.82 g, 65%): mp 104-105 °C (lit[164] mp 104-105 °C); $[\alpha]_D$ = -38.11 ° (*c* 4.2 EtOH) (lit[164] $[\alpha]_D$ = -37.8 ° (*c* 4 EtOH)); IR (nujol) 2904, 1612, 1586 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.0 (s, 3 H, CH_3CO), 2.48-2.62 (m, 2 H, CH_2CHNH), 4.40 (dd, *J* = 8.0 Hz, 5.0 Hz, *CH*NH), 5.10-5.19 (m, 2 H, CH_2=CH), 5.71-5.82 (m, 1 H, CH_2=*CH*); exact mass 157.0733 (157.0739 calcd for $C_7H_{11}NO_3$); Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.62; H, 7.14; N, 8.83.



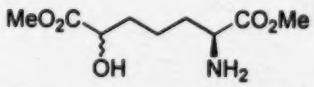
Methyl *N*-(Benzyloxycarbonyl)-L-allylglycinate (46). Diazomethane was prepared freshly as follows:[165] to a solution of 2-ethoxyethanol (32 mL) in ether (40 mL) was added a solution of KOH (6 g, 107 mmol) in water (10 mL). The mixture was brought to reflux in smooth joint distillation apparatus. A solution of diazide ($CH_3C_6H_4SO_2NCH_3NO$) (21.4 g, 100 mmol) in ether (80 mL) was added dropwise and the ethereal solution of yellow CH_2N_2 solution was collected in a 500 mL conical flask cooled to 0 °C. Compound **5** (3.28g, 13.2 mmol) was dissolved in Et_2O (20 mL) which was treated with the freshly prepared diazomethane in Et_2O. After 10 min, excess diazomethane was destroyed by the addition of 2 M HCl. The solvent was evaporated *in vacuo* to afford an oil which was purified by flash chromatography (70% EtOAc in hexane) to give **46** as an oil (3.19 g, 92%): R_f 0.68 (70% EtOAc in hexane); $[\alpha]_D$ = +13.77 (*c* 1.0 $CHCl_3$); IR ($CHCl_3$ cast) 3341, 1724, 1525 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.48-2.67 (m, 2 H, CH_2CHNH), 3.76 (s, 3 H, OCH_3), 4.44-4.52 (m, 1 H, *CH*NH),

5.08-5.20 (m, 4 H, CH_2=CH), 5.30 (d, 1 H, J = 8 Hz, N*H*), 5.60-5.75 (br m, 1 H, CH_2=C*H*), 7.30-7.42 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.70 (*C*H_2CHNH), 52.33 (*C*HNH), 53.26 (OCH_3), 66.98 (*C*H_2Ph), 119.37 (*C*H_2=CH), 128.08, 128.16, 128.50 (aromatic *C*H's), 131.97 (CH_2=*C*H), 136.22 (quaternary aromatic C), 155.68 (NH*C*O_2), 172.14 (*C*O_2CH_3); exact mass 263.1154 (7, M^+) (263.1157 calcd for $C_{14}H_{17}NO_4$); Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.94; H, 6.43; N, 5.17.



Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydroxy-4-heptene-1,7-dioate (47). The procedure of Cox et al. to make this compound was followed.[19] To a solution of methyl glyoxylate (**32**) (3.13 g, 35.6 mmol) in CH_2Cl_2 (60 mL) was added $SnCl_4$ (18.5 mL, 15.8 mmol) at -55 °C over 10 min. The solution was stirred a further 10 min and then cooled to -78 °C. A solution of **46** (3.9 g, 14.8 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 10 min. The suspension was warmed to -23 °C, stirred for 4h, then poured into ice-water (200 mL) and extracted into CH_2Cl_2 (4 x 200 mL). The combined organic extracts were dried and evaporated *in vacuo* to afford an oil which was purified by flash chromatography (70% EtOAc in hexane) to give **47** as an oil (3.4 g, 66%): R_f 0.80 (EtOAc); IR ($CHCl_3$ cast) 3462, 3358, 2955, 1743, 1522 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.35-2.60 (m, 2 H, C*H*$_2$CH=CH), 3.55 (s, 3 H, OC*H*$_3$), 3.76 (s, 3 H, OC*H*$_3$), 4.20-4.27 (m, 1 H, C*H*OH), 4.80-4.89 (m, 1 H, C*H*NH), 5.11 (s, 2 H, C*H*$_2$Ph), 5.60-5.65 (m, 1 H, C*H*=CH), 5.72-5.81 (m, 1 H, CH=C*H*), 7.29-7.39 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) 36.98 (*C*H_2CH=CH), 52.50 (OCH_3), 52.69 (OCH_3), 55.51

(*C*HNH), 67.09 (*C*H_2Ph), 69.82 (*C*HOH), 128.01, 128.11(*C*H=*C*H), 128.19, 128.24, 128.51 (aromatic *C*H's), 136.13 (quaternary aromatic C) 155.45 (NH*C*O_2), 174.45 (*C*O_2Me), 174.49 (*C*O_2Me); exact mass 351.1314 (11, M^+) (351.1318 calcd for $C_{17}H_{21}NO_7$); Anal. Calcd for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99. Found: C, 58.32; H, 6.12; N, 3.89.



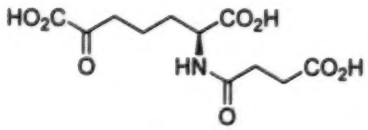
Dimethyl (6*S*)-6-Amino-2-hydroxy-heptane-1,7-dioate (48). To a solution of **47** (3.2 g, 9.1 mmol) in methanol (50 mL) was added 10% Pd/C (150 mg). The suspension was stirred under 1 atm of H_2 for 12 h then filtered through a bed of Celite and washed with methanol. The filtrate was removed *in vacuo* to give an oily residue **48** (1.95 g, 98%): IR ($CHCl_3$ cast) 3361, 3302, 1737 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.45-1.87 (m, 6 H, CH_2CH_2CH_2), 3.38-3.45 (dd, 1 H, *J* = 5.4, 4.2 Hz, C*H*N), 3.55 (s, 3 H, OCH_3), 3.73 (s, 3 H, OCH_3), 4.10-4.20 (t, 1 H, *J* = 4.2 Hz, C*H*OH); MS CI *m/z* (NH_3) 220 (21, $[M+1]^+$).

Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-oxoheptane-1,7-dioate (50). Dess-Martin reagent **49** was prepared by following the literature procedures.[167,168] Thus, to a suspension of 2-iodobenzoic acid (11.3 g, 45.2 mmol) in 100 mL solution of H_2SO_4 (7.1 g, 72.3 mmol) in H_2O was added $KBrO_3$ (9.9 g, 59 mmol) over a period of 30 min. The temperature was maintained below 55 °C by the use of an ice bath. The round-bottomed flask was then equipped with a reflux condenser and the temperature was raised

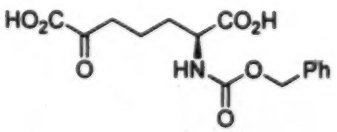
to 65 °C and stirring was maintained at this temperature for 4 h. Br_2 was given off as brown fumes which were dispersed with a stream of argon. The mixture was filtered after cooling to room temperature, and precipitate was then washed with water (100 mL), EtOH (2 x 20 mL) and ether (2 x 20 mL) to give 8.95 g of a white powder. This was added to a solution of *p*-toluenesulfonic acid (100 mg) in acetic anhydride (80 mL). The mixture was heated in an oil bath maintained at 80 °C for 2 h. The brown solution was cooled with ice and the precipitate collected by filtration to afford a white powder, which was then washed with ether (5 x 10 mL) to give **49** (5.75 g, 30%).

To a stirred solution of **23** (280 mg, 0.793 mmol) in dry CH_2Cl_2 (20 mL) under argon at room temperature was added a solution of freshly prepared Dess-Martin periodinate **49** (672 mg, 1.59 mmol) in dry CH_2Cl_2 (5 mL). After 90 min, the solution was added to a saturated aqueous solution of $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (2.0 g). The mixture was stirred vigorously for 5 min and then extracted into CH_2Cl_2 (3 x 70 mL). The combined organic layers were dried and evaporated *in vacuo,* and the residue was purified by flash chromatography (30% EtOAc in hexane) to give **50** as an oil (220 mg, 80%): R_f 0.23 (33% EtOAc in hexane); $[\alpha]_D$ = +1.08 ° (*c* 1.02 $CHCl_3$); IR ($CHCl_3$ cast) 3365, 1730, 1524 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.45-1.86 (m, 4 H, CH_2CH_2CHNH), 2.75 (t, 2 H, *J* = 6.6 Hz, MeO_2CCOCH_2), 3.62(s, 3 H, OCH_3), 3.72 (s, 3 H, OCH_3), 4.22-4.28 (m, 1 H, CHNH), 4.99 (s, 2H, CH_2Ph), 5.26 (d, 1 H, *J* = 7.2 Hz, NH), 7.15-7.26 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 18.56 (CH_2CH_2CHNH), 31.77 (CH_2CHNH), 38.49 (MeO_2CCOCH_2), 52.49 (OCH_3), 52.97 (OCH_3), 53.48 (CHNH), 67.09 (CH_2Ph), 128.11, 128.22, 128.55 (aromatic CH's), 136.16 (quaternary

aromatic C), 154.27 ($NHCO_2$), 165.32 (MeO_2CCO), 174.70 (MeO_2CCHNH), 193.34 (MeO_2CCO); CI MS *m/z* (NH_3) 369 (69, $[M+18]^+$), 352 (13, $[M+1]^+$); Anal. Calcd for $C_{17}H_{21}NO_7$: C, 58.10; H, 6.03; N, 3.99. Found: C, 57.93; H, 6.02; N, 3.88.

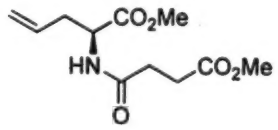


(6*S*)-2-Oxo-6-(*N*-succinylamino)heptane-1,7-dioic Acid (51). The procedure of Sherwin to make this compound was adapted.[51] Lithium hydroxide monohydrate (61.5 mg, 1.46 mmol) was added to a vigorously stirred solution of **66** (1.62 mg, 0.488 mmol) at 0 °C in H_2O (3 mL) and CH_3CN (3 mL). The resulting yellowish solution was stirred for 1 h, the CH_3CN was removed *in vacuo* and the residue was lyophilized to give **51** as a foam (91 mg, 61 %): IR (uscope) 3538, 3417, 1670, 1612 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.28-1.38 (m, 2 H, CH_2CH_2CHNH), 1.50-1.75 (m, 4 H, CH_2CHNH, CH_2CO_2H), 2.37-2.50 (m, 4 H, 2 x $COCH_2CH_2$), 3.76-3.93 (m, 1 H, CHNH); FAB MS *m/z* (Cleland) 307 (3.6, $[M+18]^+$).

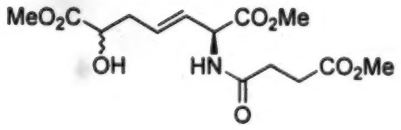


(6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-oxoheptane-1,7-dioic Acid (56). The procedure of Cox et al. was followed.[19] To **50** (129 mg, 0.369 mmol) in water (3 mL) and CH_3CN (3 mL) was added lithium hydroxide monohydrate (31.5 mg, 0.75 mmol) and the solution was stirred at ambient temperature for 90 min. Removal of solvent *in vacuo* gave a yellow residue which was dissolved in water (1 mL) and lyophilized to yield **56** (104 mg, 85%): mp 67-69 °C; IR (uscope) 3388, 2955, 1702, 1601, 1524 cm^{-1}; ^1H NMR

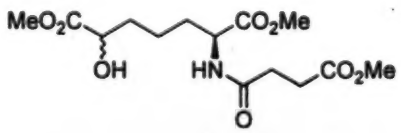
(D_2O, 300 MHz) δ 1.35-1.51 (m, 4 H, CH_2CH_2CHNH), 1.51-1.70 (m, 2 H, HO_2CCOCH_2), 3.65-3.82 (m, 1 H, $CHNH$), 4.85-5.05 (m, 2 H, CH_2Ph), 7.16-7.28 (m, 5 H, Ph); ESI MS *m/z* 346.3 (42, $[M+23]^+$).



Dimethyl *N*-Succinyl-L-allylglycinate (63). The procedure of Sherwin to make this compound was adapted.[51] Succinic anhydride (2.61 g, 26.1 mmol) was added in portions over 20 min to a saturated $NaHCO_3$ solution (70 mL) of L-allylglycine (**45**) (2.0 g, 17.4 mmol). The solution was stirred for 1 h, washed with EtOAc (30 mL), acidified with 1 M HCl to pH 1.0, and extracted into EtOAc (4 x 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo* to a residue, which was treated with an excess of an ethereal solution of CH_2N_2. Removal of solvent *in vacuo* gave **63** as a viscous oil (3.2 g, 76%): $[\alpha]_D$ = +36.2 (*c* 4.4 $CHCl_3$); IR ($CHCl_3$ cast) 3313, 3003, 1741, 1678, 1657 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.41-2.60 (m, 4 H, CH_2CO_2Me, CH_2CHNH), 2.57-2.71 (m, 2 H, $NHCOCH_2$), 3.69 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 4.66 (dt, 1 H, *J* = 6.3, 7.8 Hz, $CHNH$), 5.09-5.17 (m, 2 H, CH_2=CH), 5.62-5.75 (m, 1 H, CH_2=CH), 6.30 (d, 1 H, *J* = 7.5 Hz, NH); exact mass 243.1104 (243.1107 Calcd for $C_{11}H_{17}NO_5$); Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.17; H, 6.99; N, 5.78.

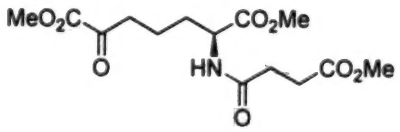


(6*S*)-2-Hydroxy-6-(*N*-succinylamino)-4-heptene-1,7-dioic Acid, Trimethyl Ester (64). The procedure of Agouridas et al. for ene reaction was adapted.[169] A stirred ice-cooled solution of freshly distilled methyl glyoxylate (**32**) (1.10 g, 12.5 mmol) in CH_2Cl_2 (20 mL) under argon was treated with anhydrous $FeCl_3$ (4.00 g, 24.6 mmol). The suspension was stirred at 0 °C for 30 min and then a solution of **63** (1.0 g, 4.1 mmol) in CH_2Cl_2 (30 mL) was added dropwise over 10 min. The suspension was warmed to 20 °C and stirred for 24 h. The black suspension was poured into ice-water (100 mL) and the mixture was extracted into CH_2Cl_2 (4 x 100 mL). The combined organic extracts were washed with 1 M HCl (100 mL), saturated $NaHCO_3$ (100 mL), then dried and evaporated *in vacuo* to give a residue which was purified by flash chromatography (70% EtOAc in hexane) to afford **64** as an oil (640 mg, 47 %): IR ($CHCl_3$ cast) 3140, 2950, 1738, 1656 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.38-2.76 (m, 6 H, 3 x CH_2), 3.68 (s, 3 H, OCH_3), 3.73 (s, 3 H, OCH_3), 4.22-4.25 (dd, 1 H, J = 6.6, 4.5 Hz, C*H*OH), 5.05 (m, 1 H, C*H*NH), 5.60-5.64 (m, 1 H, CH=C*H*), 5.72-5.82 (m, 1 H, C*H*=CH), 6.22 (br s, 1 H, N*H*); exact mass 331.1269 (331.1267 Calcd for $C_{14}H_{21}NO_8$).

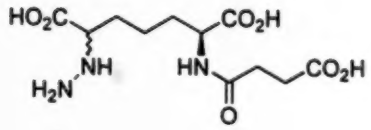


(6*S*)-2-Hydroxy-6-(*N*-succinylamino)heptane-1,7-dioic Acid, Trimethyl Ester (65). The procedure for preparation of **48** was used to convert **64** (630 mg, 1.90 mmol) to **65** in 90% yield: IR ($CHCl_3$ cast) 3140, 1738, 1656, 1531 cm^{-1}; ^1H NMR ($CDCl_3$, 360

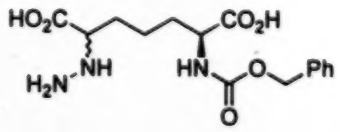
MHz) δ 1.20-1.50 (m, 2 H, $CH_2CH_2CH_2$), 1.66-1.89 (m, 4 H, $CH_2CH_2CH_2$), 2.55-2.76 (m, 4 H, $NHCOCH_2CH_2CO_2$), 3.69 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 4.15-4.20 (m, 1H, CHOH), 4.58-4.63 (m,1 H, CHNH), 6.24 (br s, 1 H, NH); exact mass 333.1414 (333.1424 Calcd for $C_{14}H_{23}NO_8$).



(6*S*)-2-Oxo-6-(*N*-succinylamino)heptane-1,7-dioic Acid, Trimethyl Ester (66). The procedure of Dess and Martin for oxidation reaction was adapted.[167,168] To a stirred solution of **65** (310 mg, 0.94 mmol) in dry CH_2Cl_2 (15 mL) under argon was added a solution of freshly prepared Dess-Martin periodinane (**49**) (645 mg, 1.5 mmol) in dry CH_2Cl_2 (10 mL). After 90 min the solution was added to a saturated solution of $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (3.0 g). The mixture was stirred vigorously for 5 min and then extracted into CH_2Cl_2 (3 x 100 mL). The extracts were dried ($MgSO_4$), evaporated *in vacuo* and purified by flash chromatography (40% EtOAc in hexane) to afford **66** as an oil (220 mg, 72%): $[\alpha]_D$ = +18.4 ° (*c* 4.76, $CHCl_3$); IR (CH_2Cl_2 cast) 3350, 2956, 1734, 1678, 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.62-1.92 (m, 4 H, CH_2CH_2CHNH), 2.47-2.65 (m, 4H, $CO(CH_2CH_2)_2CO_2Me$), 2.85 (t, 2 H, *J* = 6.9 Hz, MeO_2CCOCH_2), 3.65 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.55-4.61 (m, 1 H, CHNH), 6.26 (d, 1 H, *J* = 7.6 Hz, NH); exact mass 331.1262 (331.1267 Calcd for $C_{14}H_{21}NO_8$). Anal. Calcd for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 50.34; H, 6.46; N, 4.47.

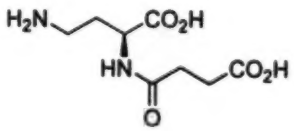


(6*S*)-2-Hydrazino-6-(*N*-succinylamino)heptane-1,7-dioic Acid (67). The procedures of Cox et al.[19] and Benigni and Dickson[170] to make this compound were adapted. To a stirred suspension of **51** (90.2 mg, 294 μmol) in anhydrous MeOH (5 mL) was added hydrazine monohydrate (143 μL, 2.94 mmol). After 60 min, $NaCNBH_3$ (184.6 mg, 2.94 mmol) was added, and the white suspension was stirred for 16 h at ambient temperature. Excess $NaCNBH_3$ was destroyed by the addition of 1 M HCl followed by stirring for another 30 min. This acidic solution was applied to BioRad AG 50 wx 8 cation exchange resin (H^+ form, 600 mL bed volume). The column was eluted with water until washings were neutral and then with 1 M aqueous NH_3. The fractions containing hydrazine compounds were pooled and evaporated *in vacuo*. The residue was suspended in water (2 mL) and lyophilized to give a pale yellow powder (45.7 mg, 51%): mp 92-94 °C; IR (uscope) 3256, 3148, 1567, 1400 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.35-1.45 (m, 2 H, $CH_2CH_2CH_2$), 1.62-1.84 (m, 4 H, CH_2CHNH, $NH_2NHCHCH_2$), 2.41-2.60 (m, 4 H, CH_2CO_2H, $COCH_2$), 3.42 (t, 1 H, J = 6.2 Hz, $CHNHNH_2$), 4.23 (dd, 1 H, J = 4.7, 6.6 Hz, CHNHsuccinyl); ESI MS *m/z* 328.1 (18, $[M+23]^+$).



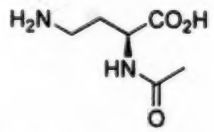
(6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-hydrazinoheptane-1,7-dioic Acid (68). The procedure used for preparation of **67** was employed to convert **56** (102 mg, 0.32 mmol) to **68** in 42% yield: mp 53-55 °C; IR (uscope): 3335, 3181, 2418, 2342, 1695,

1602 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.42 (qui, 2 H, J = 7.6 Hz, CH_2CH_2CHNH), 1.55-1.69 (m, 4 H, $CH_2CH_2CH_2$), 3.31-3.38 (m, 1 H, $CHNHNH_2$), 3.90-3.99 (m, 1 H, $CHNHCbz$), 5.08-5.18 (m, 2 H, CH_2Ph), 7.39-7.48 (m, 5 H, Ph); ^{13}C NMR (D_2O, 75.5 MHz) δ 20.12 ($CH_2CH_2CH_2$), 27.81, 29.20 ($CH_2CH_2CH_2$), 52.84 ($CHNH_2$), 59.66 ($CHNHCbz$), 66.09 (CH_2Ph), 126.70, 127.42, 127.81 (aromatic CH's), 137.25 (quaternary aromatic C), 158.72 ($NHCO_2$), 173.37 (CO_2H), 175.17 (CO_2H); ESI MS *m/z* 340.1 (83, $[M+1]^+$), 362.1 (62, $[M+23]^+$).

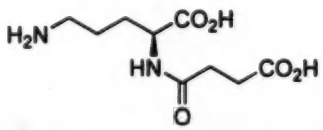


(2*S*)-4-Amino-2-(*N*-succinylamino)butyric Acid (69). A solution of (2*S*)-4-*N*-*tert*-butoxycarbonylamino-2-aminobutyric acid (500 mg, 2.29 mmol) and succinyl anhydride (343 mg, 3.43 mmol) in saturated aqueous $NaHCO_3$ (300 mL) was stirred at ambient temperature for 3 h. The resulting homogeneous solution was then washed with CH_2Cl_2 (50 mL), acidified to pH 1 with 2 M HCl, and extracted with EtOAc (4 x 200 mL). The EtOAc extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give a white powder. Without further purification, the above powder was added to a solution of TFA (15 mL) and CH_2Cl_2 (15 mL). The solution was stirred for 15 min and the solvents were evaporated *in vacuo* to give a pale yellow residue which was applied on a BioRad AG 50 Wx 8 cation exchange resin column. This was eluted with H_2O, and then with 0.5 M aqueous NH_3. The amino acid fractions were pooled and evaporated *in vacuo* to give a powder which was re-dissolved in H_2O (2 mL) and lyophilized to afford **69** (150 mg, 30%): IR (uscope): 3042, 2362, 1728, 1652, 1546, 1401 cm^{-1}; ^1H NMR (D_2O, 300 MHz)

δ 2.09 (m, 1 H, NH_2CHH) 2.28 (m, 1 H, NH_2CHH) 2.70-2.85 (m, 4 H, $COCH_2CH_2CO_2H$), 3.09 (t, 2 H, J = 8.1 Hz, CH_2CH_2CH), 4.51 (dd, 1 H, J = 5.0, 9.3 Hz, $CHNH$); ESI MS *m/z* 219.1 (100, $[M+1]^+$), 241.1 (43, $[M+23]^+$).

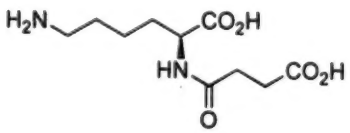


(2*S*)-2-(*N*-Acetylamino)-4-aminobutyric Acid (71). A solution of (2*S*)-2-(*N*-acetylamino)-4-(*N*-*tert*-butoxycarbonylamino)butyric acid (500 mg, 1.92 mmol) in CH_2Cl_2 (10 mL) and TFA (10 mL) was stirred at room temperature for 10 min and the solvents were evaporated *in vacuo*. The pale yellow residue was purified by ion exchange chromatography (AG50W x 8, H^+ form, 200 mL), eluting with H_2O and 2% to 10% aqueous NH_4OH to give pure **71** (182 mg, 59%): IR (uscope) 3600-2400 (br), 1671, 1541, 1432 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.08 (s, 3 H, $COCH_3$), 2.24-2.33 (m, 2 H, CH_2CHNH), 3.11 (t, 2 H, J = 8.1 Hz, NH_2CH_2), 4.50 (dd, 1 H, J = 4.8, 8.9 Hz, $CHNH$); CI MS *m/z* (NH_3) 161.1 (21, $[M+1]^+$); ESI MS *m/z* 161.1 (100, $[M+1]^+$), 183.1 (28, $[M+23]^+$).



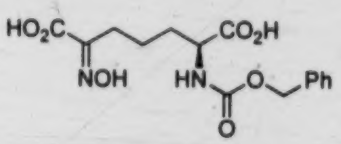
2-*N*-Succinyl-L-ornithine (72). Compound **72** was prepared in 20% yield from (2*S*)-5-*N*-*tert*-butoxycarbonylamino-2-aminopentanoic acid (90 mg, 0.388 mmol) utilizing a similar procedure as used for the synthesis of **69**: IR (uscope): 3141, 3049, 1725, 1656, 1548, 1405 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.70-2.03 (m, 4 H, CH_2CH_2CHNH), 2.58-2.71 (m, 4 H, $COCH_2CH_2CO_2H$), 3.02 (t, 2 H, J = 7.4 Hz,

NH_2CH_2), 4.42 (dd, 1 H, J = 4.7, 8.5 Hz, C*H*NH); ESI MS *m/z* 233.1 (100, $[M+1]^+$), 255.1 (69, $[M+23]^+$).

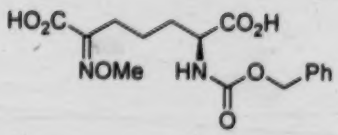


(2*S*)-6-Amino-2-(*N*-succinylamino)hexanoic Acid (74). To a stirred solution of succinic anhydride (272 mg, 2.72 mmol) and methyl 2-amino-6-(*N*-(benzyloxycarbonyl)amino)hexanoate monohydrochloride salt (600 mg, 1.814 mmol) in CH_2Cl_2 (15 mL) was added pyridine (220 μL, 2.72 mmol). The resulting solution was stirred at ambient temperature for 90 min before the solvent was removed *in vacuo*. The residue was redissolved in Et_2O (20 mL), washed with 1 M HCl (20 mL), and then saturated $NaHCO_3$ (20 mL). The $NaHCO_3$ solution was acidified to pH 2 with 1 M HCl, and extracted with Et_2O (5 x 100 mL). The combined ethereal extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give a yellowish residue. This was dissolved in a solution of H_2O (4 mL) and CH_3CN (4 mL) with lithium hydroxide monohydrate (76 mg, 1.814 mmol). After stirring for 1h at ambient temperature, the solution was evaporated *in vacuo*, and the residue was transferred to a solution of acetic acid (1 mL) and H_2O (15 mL) with 10% Pd/C (20 mg). The resultant mixture was stirred under 1 atm of H_2 at ambient temperature for 12 h, and the black Pd/C was filtered through a bed of Celite. The filtrate was evaporated *in vacuo* and the residue was applied on a BioRad 50 Wx 8 cation exchange column (H^+ form), and eluted with H_2O, followed by 1 M NH_3. The fractions containing amino acid were pooled and evaporated *in vacuo* to afford **74** (110 mg, 25%): IR (uscope) 3046, 1727, 1650, 1548, 1404 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ

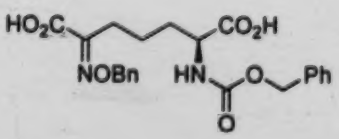
1.47 (m, 2 H, $NH_2(CH_2)_2CH_2$), 1.51-2.05 (m, 4 H, $NH_2CH_2CH_2CH_2CH_2$), 2.52-2.70 (m, 4 H, $COCH_2CH_2CO_2H$), 3.01 (t, 2 H, J = 7.5 Hz, NH_2CH_2), 4.38 (dd, 1 H, J = 5.0, 8.9 Hz, CHNH); ESI MS *m/z* 247.1 (100, $[M+1]^+$), 269.0 (16, $[M+23]^+$).



(6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(hydroxyimino)heptane-1,7-dioic Acid (77). A mixture of **83** (108 mg, 0.295 mmol) and NaOH (118 mg, 2.95 mmol) in H_2O (3 mL) was vigorously stirred for 18 h. Then another portion of NaOH (100 mg, 2.5 mmol) was added and the mixture was stirred at ambient temperature for 3 h. The resulting aqueous layer was washed with CH_2Cl_2 (10 mL), then acidified to pH 1 with 1 M HCl, and extracted with EtOAc (3 x 200 mL). The EtOAc extracts were dried over anhydrous Na_2SO_4, evaporated *in vacuo* and purified by flash chromatography (30% conc. NH_3 in *i*-PrOH) to give **77** (54 mg, 54%): R_f 0.50 (30% conc. NH_3 in *i*-PrOH); IR (uscope): 2943 (br), 1682, 1529, 1454 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 1.53-1.89 (m, 4 H, CH_2CH_2CHNH), 2.51-2.65 (m, 2 H, $N=CCH_2$), 3.95-4.08 (m, 1 H, CHNH), 5.07-5.20 (m, 2 H, CH_2Ph), 7.36-7.47 (m, 5 H, Ph); ^{13}C NMR (D_2O, 100 MHz) δ 21.83 ($CH_2CH_2CH_2$), 24.36 (CH_2CHNH), 31.50 ($N=CCH_2$), 66.64 (CH_2Ph), 127.39, 128.11, 128.62 (aromatic CH's), 157.74 (N=C), 159.14 (quaternary aromatic C), 170.53 ($MeO_2CC=$), 180.44 ($CHCO_2Me$); ESI MS *m/z* 339.1 (17, $[M+1]^+$), 361.1 (100, $[M+23]^+$).

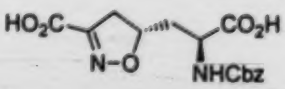


(6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(methoxyimino)heptane-1,7-dioic Acid (78). A mixture of **84** (100 mg, 0.284 mmol) and 10% aqueous NaOH solution (2 mL) was vigorously stirred for 2 h, then diluted with H_2O (6 mL) and stirred for a further 12 h. Then NaOH (850 mg) was added and the resultant mixture was stirred at ambient temperature for another 5 h. The mixture was diluted with H_2O (10 mL), washed with EtOAc (5 mL), acidified to pH 1 with 1 M HCl, and extracted with EtOAc (3 x 20 mL). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo* to give **78** as a powder (90 mg, 90%): R_f 0.68 (30% conc. NH_3 in *i*-PrOH); IR ($CHCl_3$ cast) 3331, 2924, 1719, 1530, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.76-1.92 (m, 4 H, $CH_2CH_2CH_2CH$), 2.47-2.64 (m, 2 H, N=CCH_2), 4.02 (s, 3 H, OCH_3), 4.38-4.50 (dt, 1 H, *J* = 5.3, 7.8 Hz, C*H*NH), 5.10 (s, 2 H, CH_2Ph), 5.38 (d, 1 H, *J* = 7.8 Hz, N*H*), 7.26-7.39 (m, 5 H, Ph), 9.50 (br s, 2 H, 2 x COO*H*); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.70 ($CH_2CH_2CH_2$), 23.93 (CH_2CHNH), 31.88 (N=CCH_2), 53.44 (*C*HNH), 63.68 (OCH_3), 67.27 (*C*H_2Ph), 128.12, 128.28, 128.56 (aromatic *C*H's), 136.02 (quaternary aromatic C), 150.99 (N=*C*), 156.15 ($NHCO_2$), 164.87 (HO_2CC=), 176.98 ($CHCO_2H$); ESI MS *m/z* 375.1 (53, $[M+23]^+$).



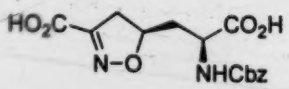
(6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(benzyloxyimino)heptane-1,7-dioic Acid (79). Compound **85** (75 mg, 0.164 mmol) in 1 M NaOH solution (5 mL) was

stirred at ambient temperature for 15 h and then CH_3CN (3 mL) was added. After 4 h, the solvents were evaporated *in vacuo*, and the residue was redissolved in H_2O (2 mL). The aqueous solution was washed with CH_2Cl_2 (2 mL), acidified to pH 1 with 1 M HCl, and extracted with EtOAc (5 x 20 mL). The combined EtOAc extracts were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give **79** (65 mg, 93%): R_f 0.67 (30% conc. NH_3 in *i*-PrOH); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 1.56-1.91 (m, 4 H, CH_2CH_2CHNH), 2.79 (t, 2 H, J = 5.2 Hz, N=CCH_2), 4.32-4.42 (m, 1 H, C*H*NH), 5.12 (s, 2 H, CO_2CH_2Ph), 5.28 (s, 2 H, =NOCH_2Ph), 5.42 (d, 1 H, J = 8.1 Hz, N*H*), 7.26-7.40 (m, 10 H, 2 x Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.77 ($CH_2CH_2CH_2$), 24.25 (CH_2CHNH), 31.89 (N=CCH_2), 53.55 (*C*HNH), 67.20 ($NHCO_2CH_2Ph$), 78.03 (=NOCH_2Ph), 128.12, 128.25, 128.35, 128.49, 128.56, 128.63 (aromatic *C*H's), 136.16 (quaternary aromatic C), 156.19 (*C*=N), 164.65 ($NHCO_2$), 175.64 (CO_2H), 176.21 (CO_2H); ESI MS *m/z* 429.2 (8, $[M+1]^+$), 451.2 (100, $[M+23]^+$).

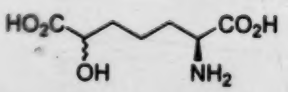


(2*S*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (80). The procedure of Abbott et al. was adapted.[23] A mixture of **127** (227 mg, 0.623 mmol) and 10% aqueous NaOH solution (2 mL) was stirred vigorously for 2 h and then diluted with H_2O (6 mL) for a further 12 h. Then another 4 g of NaOH was added and it was stirred for an additional 4 h. The mixture was diluted with H_2O (10 mL), washed with EtOAc (5 mL), acidified to pH 1 with 1 M HCl, and extracted with EtOAc (3 x 20 mL). The combined extracts were dried and evaporated *in vacuo* to give **80** (203 mg, 97%): IR ($CHCl_3$ cast) 3468, 3121, 1718, 1540, 1479 cm^{-1}; ^{1}H NMR

(acetone-d_6, 300 MHz) δ 2.05 (A of ABXY, 1 H, J = 4.0, 10.5, 14.5 Hz, CHCH_aCHNH), 2.31 (B of ABXY, 1 H, J = 4.0, 9.0, 14.5 Hz, CHCH_bCHNH), 2.96 (A of ABX, 1 H, J = 8.0, 17.5 Hz, N=CCH_a), 3.36 (B of ABX, 1 H, J = 11.0, 17.5 Hz, N=CCH_b), 4.47 (ddd, 1 H, J = 4.0, 8.5, 10.5 Hz, C*H*NH), 4.97 (dddd, 1 H, J = 4.0, 8.0, 9.0, 11.0 Hz, CH_2CHCH_2), 5.09 (s, 2 H, CH_2Ph), 6.70 (d, 1 H, J = 8.5 Hz, N*H*), 7.24-7.39 (m, 5 H, Ph); FAB MS (Cleland) *m/z* 337 (57, $[M+1]^+$).

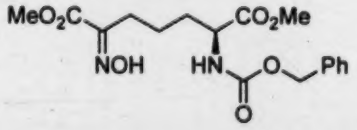


(2*S*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (81). The procedure to make **80** was employed to convert **128** (220 mg, 1.65 mmol) to **81** in 95% yield: IR ($CHCl_3$ cast) 3458, 3163, 1719, 1528, 1432 cm^{-1}; ^{1}H NMR (acetone-d_6, 300 MHz) δ 2.21 (dd, 2 H, J = 7.0, 7.0 Hz, CHCH_2CH), 3.01 (A of ABX, 1 H, J = 8.0, 17.5 Hz, N=CCH_a), 3.38 (B of ABX, 1 H, J = 11.0, 17.5 Hz, N=CCH_b), 4.40 (dt, 1 H, J = 7.0, 8.0 Hz, C*H*NH), 5.00 (ddt, 1 H, J = 7.0, 8.0, 11.0 Hz, CH_2CHCH_2), 5.09 (s, 2 H, CH_2Ph), 6.73 (d, 1 H, J = 8.0 Hz, N*H*), 7.25-7.40 (m, 5 H, Ph); FAB MS *m/z* (Cleland) 337 (70, $[M+1]^+$)



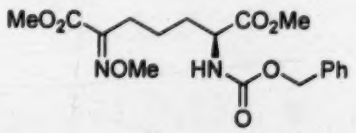
(6*S*)-6-Amino-2-hydroxyheptane-1,7-dioic Acid (82). A solution of **48** (168 mg, 0.88 mmol) and lithium hydroxide monohydrate (76 mg, 1.80 mmol) in H_2O (10 mL) and CH_3CN (10 mL) was stirred at ambient temperature for 5 h. CH_3CN was evaporated *in vacuo* and the aqueous solution was lyophilized to give a white powder. The powder was applied on a BioRad AG 50 Wx 80 cation exchange resin column (H^+ form, 100 mL

bed volume), eluted with water and then with 0.5 M aqueous NH_3 solution. The amino acid fractions were combined and evaporated *in vacuo* to give a pale yellow powder which was re-suspended in water (2 mL) and lyophilized to afford **82** (104 mg, 62%): IR (uscope) 3281, 2953, 1732, 1559 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.38-1.59 (m, 2 H, $CH_2CH_2CH_2$), 1.64-2.05 (m, 4 H, $CH_2CH_2CH_2$), 3.68-3.81 (m, 1 H, $CHNH_2$), 4.04-4.10 (m, 1 H, CHOH); CI MS *m/z* (NH_3) 192 (35, $[M+1]^+$).



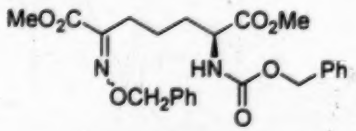
Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(hydroxyimino)heptane-1,7-dioate (83). The procedure of Corey et al. for oximine formation was adapted.[171] To a stirred solution of **50** (133 mg, 0.379 mmol) and hydroxylamine hydrochloride (42 mg, 0.606 mmol) in methanol (15 mL) was added pyridine (80 μL, 0.985 mmol) dropwise. The solution was stirred under argon for another 2 h. The solvent was removed *in vacuo*, and the residue was redissolved in EtOAc (20 mL), and washed with 1 M HCl (10 mL). The organic layer was dried ($MgSO_4$), and evaporated *in vacuo* to give a residue which was purified by flash chromatography to afford **83** as an oil (87 mg, 63%): R_f 0.30 (40% EtOAc in hexane); $[\alpha]_D$ = +2.6 ° (*c* 0.46, $CHCl_3$); IR ($CHCl_3$ cast) 3336, 3034, 2955, 1722, 1530 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.52-1.91 (m, 4 H, CH_2CH_2CHNH), 2.62 (t, 2 H, J = 7.2 Hz, $CH_2C{=}N$), 3.53 (s, 3 H, OCH_3(imine part)), 3.80 (s, 3 H, OCH_3), 4.42 (dt, 1 H, J = 5.7, 7.8 Hz, CHNH), 5.11 (s, 2 H, CH_2Ph), 5.50 (d, 1 H, J = 7.8 Hz, NH), 7.24-7.37 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.87 ($CH_2CH_2CH_2$), 24.12 (CH_2CHNH), 32.28 ($CH_2C{=}N$), 52.49 (OCH_3), 52.67 (OCH_3), 53.69 (CHNH), 67.08

(CH_2Ph), 128.11, 128.20, 128.54 (aromatic *C*H's), 136.19 (quaternary aromatic C), 152.01 (*C*=N), 156.09 ($NHCO_2$), 163.96 (MeO_2CC=N), 173.18 (CO_2Me); ESI MS *m/z* 389.1 (100, $[M+23]^+$); Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.73; H, 6.05; N, 7.65. Found: C, 55.92; H, 6.31; N, 7.75.

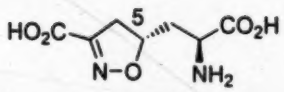


Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(methoxyimino)heptane-1,7-dioate (84). The procedure of Corey et al. for oximine formation was adapted.[171] To a stirred solution of **50** (123 mg, 0.35 mmol) and methoxylamine hydrochloride (46.8 mg, 0.561 mmol) in methanol (10 mL) was added pyridine (74 μL, 0.91 mmol) dropwise. The solution was stirred under argon for another 2 h. The solvent was removed *in vacuo* and the residue was redissolved in EtOAc (20 mL), and washed with 1 M HCl (10 mL). The organic layer was dried ($MgSO_4$), and evaporated *in vacuo* to give a residue which was purified by flash chromatography to afford **84** as an oil (100 mg, 75%): R_f 0.56 (40% EtOAc in hexane); $[\alpha]_D$ = +4.04 ° (*c* 1.36, $CHCl_3$); IR ($CHCl_3$ cast) 3361, 2952, 1723, 1586 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.46-1.89 (m, 4 H, CH_2CH_2CHNH), 2.55 (t, 1 H, *J* = 7.3 Hz, N=CCH_2), 3.70 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 4.05 (s, 3 H, OCH_3), 4.37 (dt, 1 H, *J* = 5.3, 7.8 Hz, *CH*NH), 5.09 (s, 2 H, CH_2Ph), 5.35 (d, 1 H, *J* = 7.8 Hz, *CH*NH), 7.26-7.33 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 21.89 ($CH_2CH_2CH_2$), 24.79 (CH_2CHNH), 32.29 (CH_2C=N), 52.34 (CH_3O), 52.70 (CH_3O), 53.58 (*C*HNH), 63.24 (OCH_3), 66.98 (CH_2Ph), 128.08, 128.16, 128.51 (aromatic *C*H's), 136.26 (quaternary aromatic C), 151.69 (*C*=N), 155.89 ($NHCO_2$), 163.87 (MeO_2CC=N), 172.80

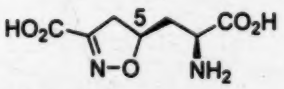
(CO_2Me); ESI MS *m/z* 403.1 (100, $[M+23]^+$); Anal. Calcd for $C_{18}H_{24}N_2O_7$: C, 56.83; H, 6.36; N, 7.36. Found: C, 56.97; H, 6.38; N, 7.28.



Dimethyl (6*S*)-6-(*N*-(Benzyloxycarbonyl)amino)-2-(benzyloxyimino)heptane-1,7-dioate (85). The procedure of Corey et al. for oximine formation was adapted.[171] To a stirred solution of **50** (138 mg, 0.393 mmol) and *o*-benzylhydroxylamine hydrochloride (100 mg, 0.629 mmol) in methanol (15 mL) was added pyridine (83 μL, 1.02 mmol) dropwise. The solution was stirred under argon for another 2 h. The solvent was removed *in vacuo*, and the residue was redissolved in EtOAc (20 mL), and washed with 1 M HCl (10 mL). The organic layer was dried ($MgSO_4$), and evaporated *in vacuo* to give a residue which was purified by flash chromatography to afford **85** as an oil (82 mg, 46%): R_f 0.55 (40% EtOAc in hexane); $[\alpha]_D$ = +4.69 ° (*c* 0.8, $CHCl_3$); IR ($CHCl_3$ cast) 3363, 2952, 1723, 1606, 1524 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.49-1.86 (m, 4 H, CH_2CH_2CHNH), 2.60 (t, 1 H, *J* = 7.3 Hz, $N{=}CCH_2$), 3.69 (s, 3 H, CH_3O), 3.85 (s, 3 H, OCH_3), 5.10 (s, 2 H, $NHCO_2CH_2Ph$), 5.29 (s, 2 H, $=NOCH_2Ph$), 7.26-7.41 (m, 10 H, 2 x Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.91 ($CH_2CH_2CH_2$), 25.12 (CH_2CHNH), 32.34 ($N{=}CCH_2$), 52.32 (CH_3O), 52.73 (OCH_3), 53.64 (*C*HNH), 67.02 ($NHCO_2CH_2Ph$), 77.75 ($=NOCH_2Ph$), 127.76, 128.11, 128.19, 128.26, 128.54 (aromatic *C*H's), 136.25, 136.51 (quaternary aromatic C's), 152.13 (*C*=N), 155.88 ($NHCO_2$), 163.95 ($MeO_2CC{=}N$), 172.74 (MeO_2CCH); ESI MS *m/z* 479.2 (100, $[M+23]^+$); Anal. Calcd for $C_{24}H_{28}N_2O_7$: C, 63.15; H, 6.18; N, 6.14. Found: C, 63.42; H, 6.39; N, 5.98.

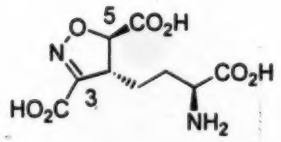


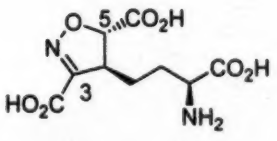
(2*S*, 5'*S*)-2-Amino-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (99). The procedure of Abbott et al. to make this compound was adapted.[23] A solution of **80** (220 mg, 0.65 mmol) and TMSCl (333 μL, 2.60 mmol) in dry CH_3CN (3 mL) under argon was treated with NaI (197 mg, 1.3 mmol), and the mixture was stirred at ambient temperature for 2 h. A second portion of NaI (197 mg, 1.3 mmol) was added. After 17 h, H_2O (5 mL) was added and the mixture was washed with CH_2Cl_2 (10 mL) and freeze-dried. The black residue was purified by flash chromatography with 30% NH_3 in *i*-PrOH to give a pale yellow solid **99** (54 mg, 41%): R_f 0.25 (30% NH_3 in *i*-PrOH); $[\alpha]_D$ = -38 ° (*c* 1.09 H_2O); IR (uscope) 2952, 1630, 1575, 1397 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 2.20 (A of ABXY, 1 H, *J* = 4.0, 6.5, 15.5 Hz, CHCH_aCHNH), 2.27 (B of ABXY, 1 H, *J* = 4.0, 9.5, 15.5 Hz, CHCH_bCHNH), 2.97 (A of ABX, 1 H, *J* = 7.0, 18.0 Hz, N=CCH_a), 3.43 (B of ABX, 1 H, *J* = 10.5, 18.0 Hz, N=CCH_b), 3.95 (dd, 1 H, *J* = 4.0, 6.5 Hz, C*H*NH), 4.92 (dddd, 1 H, *J* = 4.0, 7.0, 9.5, 10.5 Hz, CH_2CHCH_2); FAB MS (Cleland) *m/z* 203 (29, $[M+1]^+$).



(2*S*, 5'*R*)-2-Amino-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (100). The procedure to make **99** was employed to convert **81** (80 mg, 0.23 mmol) to **100** in 40% yield: $[\alpha]_D$ = +98.0 ° (*c* 0.80 H_2O); IR (KBr) 3600-3300, 3250-2500, 1620 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 2.17 (A of ABXY, 1 H, *J* = 2.5, 8.0, 15.0 Hz, CHCH_aCHNH), 2.30 (B of ABXY, 1 H, *J* = 3.0, 5.5, 15.0 Hz, CHCH_bCHNH), 2.98 (A of ABX, 1 H, *J* = 7.0, 18.0 Hz, N=CCH_a), 3.45 (B of ABX, 1 H, *J* = 10.5, 18.0 Hz, N=CCH_b), 4.04 (dd, 1 H, *J* = 5.5,

8.0 Hz, C*H*NH), 5.06-5.14 (m, 1 H, CH_2CHCH_2); FAB MS (Cleland) *m/z* 203 (74, $[M+1]^+$).

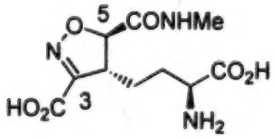


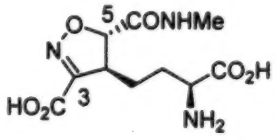


(2*S*,4'*R*, 5'*R*)-2-Amino-4-(3,5-dicarboxy-2-isoxazolin-4-yl)butanoic Acid (104a), and (2*S*, 4'*S*, 5'*S*)-2-Amino-4-(3,5-dicarboxy-2-isoxazolin-4-yl)butanoic Acid (104b). The procedure of Abbott et al. was adapted.[23] To the mixture of **118a** and **118b** (100 mg, 0.209 mmol) in H_2O (2 mL) and CH_3CN (2 mL) was added lithium hydroxide monohydrate (19.3 mg, 0.46 mmol). The resulting yellowish solution was stirred at ambient temperature for 2 h and then concentrated *in vacuo*. The residue was re-dissolved in H_2O (5 mL), acidified to pH 1 with 1 M HCl, and extracted with EtOAc (5 x 10 mL). The combined extracts were dried ($MgSO_4$), and evaporated *in vacuo* to give a mixture of **119a** and **119b** (90 mg, 96%) which was used without further purification: R_f 0.69 (30% NH_3 in *i*-PrOH); ^1H NMR (D_2O, 400 MHz) δ 1.38-1.52 (m, 9 H, $C(CH_3)_3$ of two isomers), 1.66-2.01 (m, 4 H, CH_2CH_2), 4.00 (m, 1 H, =C*H*), 5.09-5.24 (m, 3 H, O*CH*, CH_2Ph), 7.40-7.51 (m, 5 H, Ph);

The mixture of **119a** and **119b** (90 mg, 0.2 mmol) was suspended in dry CH_3CN (10 mL) and to this solution was added fresh trimethylsilyl iodide (86 μL, 0.6 mmol) solution. The colorless solution turned red immediately. After stirring at ambient temperature for 90 min, the CH_3CN was evaporated *in vacuo* and the residue was partitioned between CH_2Cl_2 (15 mL) and H_2O (10 mL). The aqueous layer was then

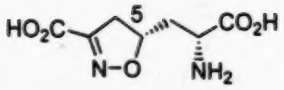
freeze-dried and the resulting black residue was purified by flash chromatography (10% NH_3, and then 40% NH_3 in *i*-PrOH) to give a mixture of **104a** and **104b** (19.2 mg, 36%): R_f 0.28 (40% NH_3 in *i*-PrOH); IR (uscope) 3049-2010 br, 1652, 1548, 1400 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.71-2.07 (m, 4 H, CH_2CH_2), 3.63 (m, 1 H, =CC*H*), 3.78 (m, 1 H, C*H*N); ESI MS *m/z* 283.0 (100, $[M+23]^+$).



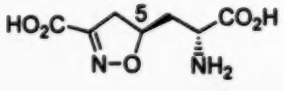


(2*S*, 4'*R*, 5'*R*)-2-Amino-4-(3-carboxy-5-(*N*-methylcarbamoyl)-2-isoxazolin-4-yl)butanoic Acid (105a), and (2*S*, 4'*S*, 5'*S*)-2-Amino-4-(3-carboxy-5-(*N*-methyl-carbamoyl)-2-isoxazolin-4-yl)butanoic Acid (105b). The procedure to make **104a** and **104b** was employed to synthesize **105a** and **105b** from **121a** and **121b** respectively. Isomer A (4.8 mg, 24% from one isomer of **121**): R_f 0.16 (30% NH_3 in *i*-PrOH); IR (uscope) 3600-2400 (br), 1651, 1574, 1451 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 1.86-1.99 (m, 4 H, CH_2CH_2), 2.79 (s, 3 H, $NHCH_3$), 3.66-3.71 (m, 1 H, =CC*H*), 3.80-3.72 (m, 1 H, C*H*N); ESI MS *m/z* 274.1 (22, $[M+1]^+$).

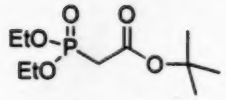
Isomer B (3.9 mg, 21% from the other isomer of **121**): R_f 0.16 (30% NH_3 in *i*-PrOH); IR (uscope) 3600-2600 (br), 1658, 1549, 1406 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 2.30-2.05 (m, 4 H, CH_2CH_2), 2.80 (s, 3 H, CH_3), 3.67-3.81 (m, 2 H, =CC*H*, C*H*N); CI MS *m/z* (NH_3) 291 (33, $[M+18]^+$).



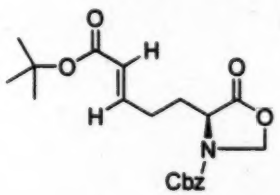
(2*R*, 5'*S*)-2-Amino-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (106). The procedure to make **99** was employed to convert **125** (260 mg, 0.77 mmol) to **106** in 44% yield: R_f 0.15 (30% NH_3 in *i*-PrOH); $[\alpha]_D$ = -82.0 ° (*c* 0.43 H_2O); IR (uscope) 2952, 1575, 1397 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 2.17 (A of ABXY, 1 H, *J* = 2.5, 8.0, 15.0 Hz, CHCH_aCHNH), 2.30 (B of ABXY, 1 H, *J* = 3.0, 5.5, 15.0 Hz, CHCH_bCHNH), 2.96 (A of ABX, 1 H, *J* = 6.6, 18.0 Hz, N=CCH_a), 3.45 (B of ABX, 1 H, *J* = 10.5, 18.0 Hz, N=CCH_b), 3.93 (dd, 1 H, *J* = 5.1, 8.4 Hz, C*H*NH), 5.06-5.14 (m, 1 H, CH_2CHCH_2); CI MS (NH_3) *m/z* 203 (79, $[M+1]^+$).



(2*R*, 5'*R*)-2-Amino-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (107). The procedure to make **99** was employed to convert **126** (188 mg, 0.56 mmol) to **107** in 51% yield: R_f 0.15 (30% NH_3 in *i*-PrOH); $[\alpha]_D$ = +35 ° (*c* 0.81 H_2O); IR (uscope) 3042, 1721, 1648, 1550, 1401 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 2.26 (A of ABXY, 1 H, *J* = 4.0, 6.5, 15.5 Hz, CHCH_aCHNH), 2.29 (B of ABXY, 1 H, *J* = 4.0, 9.5, 15.5 Hz, CHCH_bCHNH), 3.00 (A of ABX, 1 H, *J* = 7.0, 18.0 Hz, N=CCH_a), 3.44 (B of ABX, 1 H, *J* = 10.5, 18.0 Hz, N=CCH_b), 3.95 (dd, 1 H, *J* = 4.0, 6.5 Hz, C*H*NH), 4.92 (dddd, 1 H, *J* = 4.0, 7.0, 9.5, 10.5 Hz, CH_2CHCH_2); ESI MS *m/z* 203.1 (80, $[M+1]^+$), 225.1 (45, $[M+23]^+$).



***tert*-Butyl Diethylphosphonoacetate (113).** The procedure of Corbel et al. was adapted.[172] *tert*-Butyl bromoacetate (19.5 g, 100 mmol) was added in portions over 5 min to a solution of triethyl phosphite (16.6 g, 100 mmol) in refluxing toluene (100 mL). After 3 h, the solution was cooled to room temperature. The solution was washed with H_2O (20 mL), dried and the solvent was removed *in vacuo* to give **113** as a yellow oil (20 g, 79%): IR ($CHCl_3$ cast) 2981, 1730, 1479, 1456, 1288 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.28 (dt, 6 H, J = 4.8, 7.1 Hz, CH_2CH_3), 1.51 (s, 9 H, $C(CH_3)_3$), 3.36 (br s, 2 H, CH_2CO), 4.08 (m, 4 H, CH_2CH_3); ESI MS *m/z* 253.1 (4, $[M+1]^+$), 275.1 (100, $[M+23]^+$).



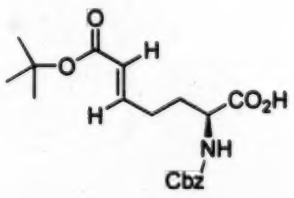
***tert*-Butyl (4*S*)-*trans*-5-(3-*N*-(Benzyloxycarbonyl)-5-oxazolidinon-4-yl)-2-pentenoate (114).** The literature procedures for Wittig reaction were adapted.[172-174] To a solution of **14** (7.2 g, 24.6 mmol) in dry benzene (40 mL) at 0 °C was added PCl_5 (5.8 g, 27.8 mmol). The mixture was stirred at 0 °C for 30 min and then at ambient temperature for 2 h, during which time it became homogeneous. The solvent was removed *in vacuo* and $POCl_3$ byproduct was azeotropically removed with dry toluene (2 x 25 mL) to give a yellow oil, which was placed under high vacuum for 48 h to afford an amorphous solid (4*S*)-3-(*N*-(benzyloxycarbonyl)-5-oxazolidinon-4-yl)propanoyl chloride (**111**) (5.79 g, 75%): IR ($CHCl_3$ cast) 2980, 1800, 1710, 1400, 1350, 1130 cm^{-1}; ^1H NMR ($CDCl_3$, 200

MHz) δ 2.24 (m, 2 H, CH_2CH_2CH), 3.05 (t, 2 H, J = 6.8 Hz, $ClOCCH_2$), 4.34 (t, 1 H, J = 6.5 Hz, NC*H*CO), 5.20 (s, 2 H, CH_2Ph), 5.22 (s, 1 H, NCH_aO), 5.52 (s, 1 H, NCH_bO), 7.38-7.42 (m, 5 H, Ph); exact mass 275.0795 (M^+ - HCl) (275.0794 calcd for $C_{14}H_{13}NO_5$).

Without further purification, **111** (5.78 g, 18.5 mmol) was dissolved in dry THF (40 mL), and the mixture was cooled to -78 °C by dry ice/*i*-PrOH bath. A suspension of $LiAl(O\text{-}t\text{-}Bu)_3H$ (4.7 g, 18.5 mmol) in dry THF (80 mL) was added dropwise (4 h, syringe pump) and the resultant mixture was then stirred at -78 °C for another 4 h. Water (20 mL) was added while the temperature was maintained at -78 °C. After 20 min the solution was allowed to warm to room temperature and the THF was removed *in vacuo*. The aqueous slurry was mixed with CH_2Cl_2 (80 mL), and the mixture was filtered through a bed of Celite. The filtrate was separated, and the organic phase was washed with H_2O (20 mL), 5% aqueous $NaHCO_3$ solution (20 mL), and dried. Concentration *in vacuo* gave an unstable oil, (4*S*)-3-*N*-(benzyloxycarbonyl)-4-(3-oxopropyl)-5-oxazolidinone (**112**) (3.59 g, 70%), which could be used without further purification: IR (uscope) 3033, 2924, 2854, 1800, 1717, 1586, 1498 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.10-2.70 (m, 4 H, CH_2CH_2), 4.38 (t, 1 H, J = 6.0 Hz, NC*H*CO), 5.22 (m, 3 H, OCH_aN, CH_2Ph), 5.55 (m, 1 H, OCH_b), 7.36 (m, 5 H, Ph), 9.68 (s, 1 H, C*H*O); exact mass 277.0947 (277.0950 calcd for $C_{14}H_{15}NO_5$).

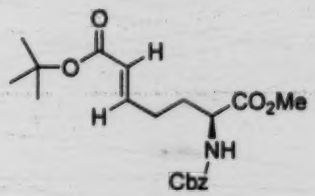
To a suspension of NaH (184 mg, 7.75 mmol) in dry THF (100 mL) was added a solution of **113** (1.96 g, 7.75 mmol) in THF (35 mL). H_2 was evolved and the solution turned yellow. After stirring at ambient temperature for another 30 min, a solution of freshly prepared **112** (2.15 g, 7.75 mmol) in dry THF (70 mL) was added dropwise within

30 min. The resulting solution was stirred at ambient temperature for 7 h, and then the solvent was evaporated *in vacuo*. The residue was redissolved in EtOAc (300 mL), and this was washed with 1 M HCl (30 mL), saturated $NaHCO_3$ (30 mL) and evaporated *in vacuo*. The yellowish residue was purified by flash chromatography (15% EtOAc in hexane) to give **114** as an oil (1.12 g, 38%): R_f 0.69 (30% EtOAc in hexane); IR ($CHCl_3$ cast): 2977, 1802, 1715, 1653, 1587, 1499 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.46 (s, 9 H, $C(CH_3)_3$), 2.02-2.39 (m, 4 H, CH_2CH_2), 4.34 (t, 1 H, J = 5.4 Hz, CHN), 5.14-5.26 (m, 3 H, CH_2Ph, NCH_aO), 5.50 (s, 1 H, NCH_bO), 5.77 (d, 1 H, J = 15.6 Hz, $CHCO_2tBu$), 6.78 (dt, 1 H, J = 8.7, 15.6 Hz, $CH{=}CHCH_2$), 7.33-7.41 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 26.89 (CH_2CHN), 28.16 ($C(CH_3)_3$), 29.13 ($=CHCH_2$), 54.32 (CHN), 68.13 (CH_2Ph), 77.92 (NCH_2O), 80.34 ($C(CH_3)_3$), 124.29 ($CHCO_2tBu$), 128.41, 128.79 (aromatic CH's), 135.32 (quaternary aromatic C), 144.87 ($CH{=}CHCH_2$), 152.87 (NCO_2), 165.57 (CO_2tBu), 171.80 (CO_2CH_2); ESI MS *m/z* 398.2 (100, $[M+23]^+$); Anal. Calcd for $C_{20}H_{25}NO_6$: C, 63.99; H, 6.71; N, 3.73. Found: C, 64.31; H, 6.94; N, 3.75.



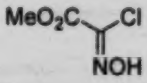
***tert*-Butyl (6*S*)-*trans*-6-(*N*-(Benzyloxycarbonyl)amino)-6-carboxy-2-hexenoate (115).** To a solution of **114** (820 mg, 2.18 mmol) in H_2O (20 mL) and CH_3CN (10 mL) was added lithium hydroxide monohydrate (120 mg, 2.44 mmol). The mixture was stirred at ambient temperature for 1 h and the solvents were evaporated *in vacuo*. The

residue was re-dissolved in H_2O (20 mL), acidified to pH 2 with 1 M HCl, and then extracted with EtOAc (5 x 50 mL). The extracts were combined, dried and evaporated *in vacuo* to give a yellow oil **115** (681 mg, 86%): R_f 0.85 (30% NH_3 in *i*-PrOH); IR (uscope) 2932, 1685, 1533 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.12 (s, 9 H, $C(CH_3)_3$), 1.57 (ddt, 1 H, J = 7.4, 9.3, 13.8 Hz, C*H*HCHN), 1.75 (ddt, 1 H, J = 4.6, 7.4, 13.8 Hz, C*H*HCHN), 2.09 (m, 2 H, =CHCH_2), 3.74 (dd, 1 H, J = 4.6, 9.3 Hz, C*H*N), 4.90 (m, 2 H, CH_2Ph), 5.57 (d, 1 H, J = 15.6 Hz, C*H*CO_2tBu), 6.71 (dt, 1 H, J = 8.7, 15.6 Hz, =C*H*CH_2), 7.18-7.27 (m, 5 H, Ph); ESI MS *m/z* 386.2 (71, $[M+23]^+$).

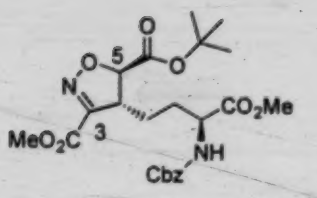


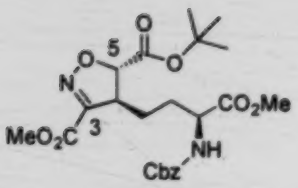
6-(*N*-(Benzyloxycarbonyl)amino)-2-heptene-1,7-dioic Acid, 1-*tert*-Butyl 7-Methyl Ester (116). The procedure to make **46** was employed to transform **115** (667 mg, 1.83 mmol) to **116** (685 mg, 99%) by the addition of diazomethane: R_f 0.61 (30% EtOAc in hexane); $[\alpha]_D$ = +16.32 ° (*c* 0.76 $CHCl_3$); IR ($CHCl_3$ cast) 3340, 1713, 1652, 1527, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.43 (s, 9 H, $C(CH_3)_3$); 1.80 (ddt, 1 H, J = 6.0, 7.8, 14.7 Hz, C*H*HCHN), 2.01 (ddt, 1 H, J = 5.1, 6.0, 14.7 Hz, C*H*HHN), 2.22 (m, 2 H, =CHCH_2), 3.73 (s, 3 H, OCH_3), 4.38 (ddd, 1 H, J = 5.1, 7.8, 7.8 Hz, C*H*N), 5.11 (s, 2 H, CH_2Ph), 5.36 (d, 1 H, J = 7.8 Hz, N*H*), 5.75 (d, 1 H, J = 15.6 Hz, C*H*CO_2tBu), 7.78 (dt, 1 H, J = 6.7, 15.6 Hz, =C*H*CH_2), 7.26-7.39 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 27.69 (*C*H_2CHN), 28.15 ($C(CH_3)_3$), 31.19 (=CH*C*H_2), 52.51 (OCH_3), 53.44 (*C*HN), 67.13

(CH_2Ph), 80.27 ($C(CH_3)_3$), 124.13 ($CHCO_2tBu$), 128.14, 128.26, 128.58 (aromatic *C*H's), 136.18 (quaternary aromatic C), 145.45 (=$CHCH_2$), 155.83 (NCO_2), 165.70 (CO_2tBu), 172.46 (CO_2Me); ESI MS *m/z* 378.1 (11, $[M+1]^+$), 400.1 (100, $[M+23]^+$); Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.78; H, 7.22; N, 3.81.

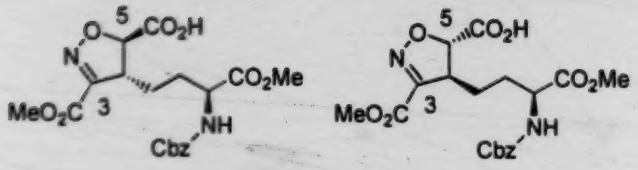


Methyl Chlorooximidoacetate (117). The literature procedures were adapted.[23,175] A cooled solution of methyl glycinate hydrochloride (2.0 g, 16 mmol) in H_2O (10 mL) was treated with concentrated HCl (5 mL) and then dropwise in 10 min with an aqueous solution (5 mL) of $NaNO_2$ (1.10 g, 16 mmol). The resulting solution was stirred at 0 °C for 10 min and then treated with a second equivalent each of concentrated HCl and aqueous $NaNO_2$. After 45 min at 0 °C, the mixture was treated with saturated aqueous NaCl (25 mL) and then extracted with Et_2O (3 x 50 mL). The combined organic extracts were dried and evaporated *in vacuo*. The solid residue was recrystalized from Et_2O/pentane to give **117** as colorless prisms (1.24 g, 57%): mp 61-63 °C (lit[175] mp 57-60 °C) ; IR (uscope) 3254, 3013, 1745, 1605, 1520 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.90 (s, 3 H, CH_3), 10.27 (br s, 1 H, NO*H*); exact mass 136.9883 and 138.9855 (55% and 18%, M^+) (136.9880 calcd for $C_3H_4{}^{35}ClNO_3$, 138.9851 calcd for $C_3H_4{}^{37}ClNO_3$); Anal. Calcd for $C_3H_4ClNO_3$: C, 26.20; H, 2.93; N, 10.18. Found: C, 26.30; H, 2.72; N, 9.97.

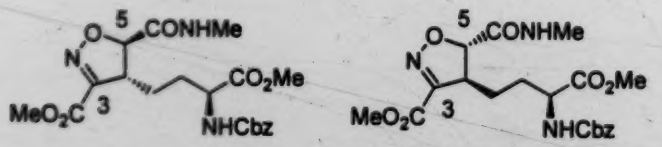




Methyl (2*S*, 4'*R*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-(*tert*-butoxycarbonyl)-3-(methoxycarbonyl)-2-isoxazolin-4-yl)butanoate (118a), and Methyl (2*S*, 4'*S*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-(*tert*-butoxycarbonyl)-3-(methoxycarbonyl)-2-isoxazolin-4-yl)butanoate (118b). The procedure to make **127** and **128** was used to generate a mixture of **118a** and **118b** (280 mg, 34%) from **116**: R_f 0.13 (single spot, 20% EtOAc in hexane) (attempts to separate these two isomers under various conditions failed); IR ($CHCl_3$ cast) 3363, 1726, 1589, 1521, 1442 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.40-1.45 (2 x s, 9 H, $C(CH_3)_3$ of two isomers), 1.58-2.03 (m, 4 H, CH_2CH_2), 3.65-3.77 (m, 3 H, $CHCO_2CH_3$ of two isomers; 0.69 H, =C*H* of isomer A), 4.84-4.90 (2 x s, 3 H, $=CCO_2CH_3$ of two isomers), 4.38-4.44 (m, 1 H, NC*H*), 4.70 (d, 0.69 H, J = 5.3 Hz, N*H* of isomer A), 4.80-4.85 (m, 0.31 H, =C*H* of isomer B), 5.12 (2 x s, 2 H, CH_2Ph of two isomers), 5.18 (d, 0.31 H, J = 4.6 Hz, N*H* of isomer B), 5.20-5.28 (m, 0.31 H, OC*H* of isomer B), 5.29-5.43 (m, 0.69 H, OC*H* of isomer A), 7.28-7.37 (m, 5 H, Ph) (from ^1H NMR the ratio of two isomers is: 0.69/0.31); ESI MS *m/z* 501.2 (100, $[M+23]^+$); Anal. Calcd for $C_{23}H_{30}N_2O_9$: C, 57.73; H, 6.32; N, 5.85. Found: C, 57.61; H, 6.22; N, 5.96.



Methyl (2*S*, 4'*R*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-carboxy-3-(methoxycarbonyl)-2-isoxazolin-4-yl)butanoate (120a), and Methyl (2*S*, 4'*S*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-carboxy-3-(methoxycarbonyl)-2-isoxazolin-4-yl)butanoate (120b). A solution of the mixture of **118a** and **118b** (190 mg, 0.40 mmol) in TFA (5 mL) was stirred at ambient temperature for 5 min. The solvent was evaporated *in vacuo* and the excess TFA was azeotropically removed with dry CH_2Cl_2 (3 x 10 mL) to give **120a** and **120b** as an oil (142 mg, 85%): IR (uscope) 3350, 3034, 1728, 1589, 1530, 1442 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.46-2.20 (m, 4 H, CH_2CH_2), 3.62-3.81 (m, 3 H, $=CCO_2CH_3$ of two isomers; 0.69 H, =CC*H* of isomer A), 3.81-3.91 (2 x s, 3 H, $CHCO_2CH_3$ of two isomers), 4.17-4.42 (m, 1 H, C*H*N), 4.91-5.00 (m, 0.31 H, =CC*H* of isomer B), 5.11 (s, 2 H, CH_2Ph), 5.17-5.27 (m, 0.31 H, OC*H* of isomer B), 5.49-5.58 (m, 1 H, OC*H* of isomer A), 7.26-7.39 (m, 5 H, Ph); ESI MS *m/z* 423.2 (28, $[M+1]^+$), 445.2 (97, $[M+23]^+$).

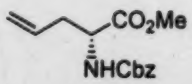


Methyl (2*S*, 4'*R*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-(*N*-methylcarbamoyl)-3-(methoxycarbonyl)-2-isoxazolin-4-yl)butanoate (121a), and Methyl (2*S*, 4'*S*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-(5-(*N*-methylcarbamoyl)-3-

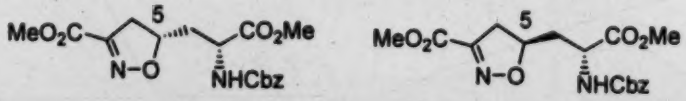
(methoxy-carbonyl)-2-isoxazolin-4-yl)butanoate (121b). To **120a** and **120b** (92 mg, 0.22 mmol) in CH_2Cl_2 (10 mL) was added (benzotriazole-1-yl-oxy)-trispyrrolidino-phosphonium hexafluorophosphate (Py-Bop), and Et_3N (61 μL, 0.44 mmol). After the solution was stirred at ambient temperature for 2 h, methylamine hydrochloride (18 mg, 0.264 mmol) was added with a second portion of Et_3N (46 μL, 0.33 mmol) in CH_2Cl_2 (10 mL). The resultant solution was stirred for another 2 h before the solvent was removed *in vacuo*. The residue was partitioned between EtOAc (20 mL) and 1 M HCl (20 mL). The organic layer was washed with saturated $NaHCO_3$ (10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Two isomers (**121a** and **121b**) were separated by flash chromatography. The isomer A (36 mg, 41%): R_f 0.34 (40% EtOAc in hexane); IR($CHCl_3$ cast) 3334, 2922, 1725, 1669, 1589, 1537, 1441 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.57-1.81 (m, 2 H, =CCHCH_2), 1.92-2.05 (m, 2 H, CH_2CHN), 2.81 (d, 3 H, J = 5.0 Hz, NHCH_3), 3.77 (s, 3 H, CHCO$_2$CH_3; m, 1 H, =CC*H*), 3.89 (s, 3 H, =CCO$_2$CH_3), 4.37-4.42 (m, 1 H, C*H*N), 4.76 (dd, 1 H, J = 4.1, 9.6 Hz, OC*H*), 5.16 (s, 2 H, CH_2Ph), 5.42 (2 x d, 1 H, J = 8.2 Hz, N*H*), 6.51-6.62 (br s, 1 H, N*H*), 7.28-7.38 (m, 5 H, Ph); ESI MS *m/z* 436.3 (1, $[M+1]^+$), 458.2 (100, $[M+23]^+$); Anal. Calcd for $C_{20}H_{25}N_3O_8$: C, 55.17; H, 5.79; N, 9.65. Found: C, 55.44; H, 5.81; N, 9.66.

Isomer B (33 mg, 37%): R_f 0.29 (40% EtOAc in hexane); IR ($CHCl_3$ cast) 3329, 2922, 1724, 1668, 1586, 1533, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.63-2.16 (m, 4 H, CH_2CH_2), 2.82 (d, 3 H, J = 5.0 Hz, NHCH_3), 3.71-3.84 (s, 3 H, OCH_3; m, 1 H, =CC*H*), 3.85-3.91 (2 x s, 3 H, OCH_3), 4.31-4.50 (m, 1 H, C*H*N), 5.10 (s, 2 H, CH_2Ph), 5.33-5.50 (m, 2 H, OC*H*, N*H*), 6.71 (br s, 1 H, N*H*), 7.28-7.38 (m, 5 H, Ph); ESI MS *m/z* 436.3 (5,

[M+1]$^+$), 458.2 (100, [M+23]$^+$); Anal. Calcd for $C_{20}H_{25}N_3O_8$: C, 55.17; H, 5.79; N, 9.65. Found: C, 55.51; H, 5.97; N, 9.69.



Methyl *N*-(Benzyloxycarbonyl)-D-allylglycinate (122). The procedure to make **46** was employed to convert **139** (920 mg, 3.69 mmol) to **122** in 85% yield: R_f 0.68 (70% EtOAc in hexane); $[\alpha]_D$ = -13.77 (*c* 1.0 $CHCl_3$); IR (uscope) 3342, 3033, 1724, 1586, 1529 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.48-2.67 (m, 2 H, C*H*$_2$CHNH), 3.76 (s, 3 H, OC*H*$_3$), 4.48 (ddd, 1 H, *J* = 4.4, 6.2, 8.0 Hz, C*H*NH), 5.08-5.20 (m, 4 H, C*H*$_2$=CH, CH_2Ph), 5.30 (d, 1 H, *J* = 8 Hz, N*H*), 5.60-5.75 (br s, 1 H, CH_2=C*H*), 7.30-7.42 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 36.70 (*C*H$_2$CHNH), 52.33 (*C*HNH), 53.26 (OC*H*$_3$), 66.98 (*C*H$_2$Ph), 119.37 (*C*H$_2$=CH), 128.08, 128.16, 128.50 (aromatic *C*H's), 131.97 (CH_2=*C*H), 136.22 (quaternary aromatic C), 155.68 (NH*C*O$_2$Ph), 172.14 (*C*O$_2$CH$_3$); ESI MS *m/z* 286.1 (100, [M+23]$^+$); CI MS *m/z* (NH_3) 264 (51, [M+1]$^+$); Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.99; H, 6.40; N, 5.17.

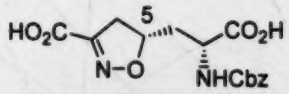


Methyl (2*R*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)propanoate (123), and Methyl (2*R*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)propanoate (124). The procedure to make **127** and **128** was employed to afford **123** (249 mg, 25%) and **124** (329 mg, 33%) as separated two compounds from **122** (723 mg, 2.75 mmol): **123**: R_f 0.45

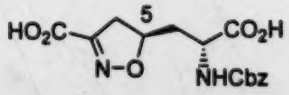
(40% EtOAc in hexane); $[\alpha]_D$ = -80 °(*c* 0.56 CH_2Cl_2); IR (uscope) 3362, 1723, 1525, 1441 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.20 (t, 2 H, *J* = 6.2 Hz, CHCH_2CH), 2.89 (A of ABX, 1 H, *J* = 7.6, 18.0 Hz, N=CCH_a), 3.32 (B of ABX, 1 H, *J* = 10.9, 17.5 Hz, N=CCH_b), 3.74 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 4.43 (dt, 1 H, *J* = 6.2, 7.0 Hz, C*H*NH), 4.89 (ddt, 1 H, *J* = 7.0, 7.5, 11.0 Hz, CH_2C*H*CH_2), 5.08 (s, 2 H, CH_2Ph), 5.66 (d, 1 H, *J* = 7.0 Hz, N*H*), 7.26-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.67 (CH*C*H_2CH), 39.06 (N=*C*CH_2), 51.34 (NH*C*H), 52.84 (O*C*H_3), 67.20 (*C*H_2Ph), 80.16 (*C*HO), 128.14, 128.31, 128.59 (aromatic *C*H's), 136.06 (quaternary aromatic C), 151.26 (*C*=N), 155.86 (O*C*ON), 160.94 (*C*OC=N), 171.68 (CH*C*O_2Me); ESI MS *m/z* 387.1 (100, $[M+23]^+$); Anal. Calcd for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 56.37; H, 5.29; N, 7.97.

For **124**: R_f 0.40 (40% EtOAc in hexane); $[\alpha]_D$ = +75 ° (*c* 0.60 CH_2Cl_2); IR (uscope): 3359, 1724, 1525, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.05 (A of ABXY, 1 H, *J* = 4.5, 8.0, 14.5 Hz, CHCH_aCHNH), 2.23 (B of ABXY, 1 H, *J* = 4.5, 9.0, 14.5 Hz, CHCH_bCHNH), 2.84 (A of ABX, 1 H, *J* = 8.0, 17.5 Hz, N=CCH_a), 3.29 (B of ABX, 1 H, *J* = 11.0, 17.5 Hz, N=CCH_b), 3.72 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.53 (ddd, 1 H, *J* = 4.5, 8.0, 8.5 Hz, C*H*NH), 4.88 (dddd, 1 H, *J* = 4.5, 8.0, 9.0, 11.0 Hz, CH_2C*H*CH_2), 5.12 (s, 2 H, CH_2Ph), 5.59 (d, 1 H, *J* = 8.5 Hz, N*H*), 7.26-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 37.75 (CH*C*H_2CH), 39.11 (N=*C*CH_2), 52.74 (NH*C*H), 52.86 (O*C*H_3), 67.22 (*C*H_2Ph), 80.64 (*C*HO), 128.14, 128.26, 128.57 (aromatic *C*H's), 136.10 (quaternary aromatic C), 151.33 (*C*=N), 155.95 (O*C*ON), 160.91 (*C*OC=N), 171.84 (CH*C*O_2Me); ESI MS *m/z* 365.2 (15, $[M+1]^+$), 387.1 (100, $[M+23]^+$);

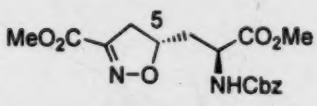
Anal. Calcd for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 55.77; H, 5.49; N, 7.96.

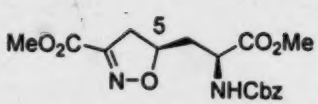


(2*R*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (125). The procedure to make **80** was employed to convert **123** (249 mg, 0.68 mmol) to **125** in 83% yield: IR (uscope) 2959, 1715, 1652, 1587, 1418 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 2.21 (dd, 2 H, J = 7.0, 7.0 Hz, CHCH_2CH), 3.01 (A of ABX, 1 H, J = 8.0, 17.5 Hz, N=CCH_a), 3.38 (B of ABX, 1 H, J = 11.0, 17.5 Hz, N=CCH_b), 4.40 (dt, 1 H, J = 7.0, 8.0 Hz, C*H*NH), 5.00 (ddt, 1 H, J = 7.0, 8.0, 11.0 Hz, CH_2CHCH_2), 5.09 (s, 2 H, CH_2Ph), 6.73 (d, 1 H, J = 8.0 Hz, N*H*), 7.25-7.40 (m, 5 H, Ph); CI MS *m/z* (NH_3) 337 (62, $[M+1]^+$).



(2*R*, 5'*R*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-carboxy-2-isoxazolin-5-yl)propanoic Acid (126). The procedure to make **80** was employed to convert **124** (329 mg, 0.90 mmol) to **126** in 87% yield: IR (uscope) 3471, 1719, 1541, 1480 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 2.05 (A of ABXY, 1 H, J = 4.0, 10.5, 14.5 Hz, CHCH_aCHNH), 2.31 (B of ABXY, 1 H, J = 4.0, 9.0, 14.5 Hz, CHCH_bCHNH), 2.96 (A of ABX, 1 H, J = 8.0, 17.5 Hz, N=CCH_a), 3.36 (B of ABX, 1 H, J = 11.0, 17.5 Hz, N=CCH_b), 4.47 (ddd, 1 H, J = 4.0, 8.5, 10.5 Hz, C*H*NH), 4.97 (dddd, 1 H, J = 4.0, 8.0, 9.0, 11.0 Hz, CH_2CHCH_2), 5.09 (s, 2 H, CH_2Ph), 6.70 (d, 1 H, J = 8.5 Hz, N*H*), 7.24-7.39 (m, 5 H, Ph); CI MS (NH_3) *m/z* 337 (64, $[M+1]^+$).

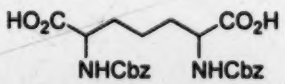




Methyl (2*S*, 5'*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)propanoate (127), and Methyl (2*S*, 5'*R*)-2-(*N*-(Benzyl-oxycarbonyl)amino)-3-(3-(methoxycarbonyl)-2-isoxazolin-5-yl)propanoate (128). The procedure of Abbott et al. was adapted.[23] A solution of methyl *N*-(benzyloxycarbonyl)-L-allylglycinate (**46**) (500 mg, 1.90 mmol) and **117** (780 mg, 5.68 mmol) in Et_2O (5 mL) was treated dropwise (3h, syringe pump) with a solution of Na_2CO_3 (603 mg, 5.69 mmol) in H_2O (4 mL) with vigorous stirring. The mixture was then stirred at ambient temperature for a further 4 h. It was diluted with Et_2O (5 mL), the phases were separated and the aqueous phase was extracted with Et_2O (3 x 20 mL). The combined ethereal layers were dried and evaporated *in vacuo*. The separation of the residue was done by flash chromatography (20% EtOAc in hexane) to give **127** (270 mg, 39%) and **128** (235 mg, 34%). For **128**: R_f 0.21 (40% EtOAc in hexane); $[\alpha]_D$ = +95.8 °(*c* 0.98 CH_2Cl_2); IR (CH_2Cl_2 cast) 3440-3275, 1722, 1524, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.17 (dd, 2 H, *J* = 6.0, 7.0 Hz, CHCH_2CH), 2.87 (A of ABX, 1 H, *J* = 7.5, 18.0 Hz, N=CCH_a), 3.32 (B of ABX, 1 H, *J* = 11.0, 17.5 Hz, N=CCH_b), 3.74 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 4.43 (dt, 1 H, *J* = 6.0, 7.0 Hz C*H*NH), 4.89 (ddt, 1 H, *J* = 7.0, 7.5, 11.0 Hz, CH_2CHCH_2), 5.08 (s, 2 H, CH_2Ph), 5.66 (d, 1 H, *J* = 7.0 Hz, N*H*), 7.26-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.30 ($CHCH_2CH$), 38.77 (N=CCH_2), 51.10 (NH*C*H), 52.59 (OCH_3), 53.13 (OCH_3), 66.91 (CH_2Ph), 80.04 (*C*HO), 127.88, 128.04, 128.34 (aromatic *C*H's), 135.89 (quaternary aromatic C), 151.08 (*C*=N), 155.75 (O*C*ON), 160.72 (*C*OC=N), 171.57 ($CHCO_2$Me); exact mass 364.1246 (364.1271 calcd

for $C_{17}H_{20}N_2O_7$). Anal. Calcd for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 56.26; H, 5.37; N, 7.72.

For **127**: R_f 0.15 (40% EtOAc in hexane); $[\alpha]_D$ = -100.9 ° (*c* 1.04 CH_2Cl_2); IR (CH_2Cl_2 cast): 3450-3270, 1724, 1525, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 2.00 (A of ABXY, 1 H, *J* = 4.5, 8.0, 14.5 Hz, CHC*H*$_a$CHNH), 2.21 (B of ABXY, 1 H, *J* = 4.5, 9.0, 14.5 Hz, CHC*H*$_b$CHNH), 2.84 (A of ABX, 1 H, *J* = 8.0, 17.5 Hz, N=CC*H*$_a$), 3.29 (B of ABX, 1 H, *J* = 11.0, 17.5 Hz, N=CC*H*$_b$), 3.72 (s, 3 H, OC*H*$_3$), 3.85 (s, 3 H, OC*H*$_3$), 4.53 (ddd, 1 H, *J* = 4.5, 8.0, 8.5 Hz, C*H*NH), 4.88 (dddd, 1 H, *J* = 4.5, 8.0, 9.0, 11.0 Hz, CH_2CHCH_2), 5.08 (s, 2 H, C*H*$_2$Ph), 5.61 (d, 1 H, *J* = 8.5 Hz, N*H*), 7.26-7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.16 (CH*C*H$_2$CH), 38.72 (N=C*C*H$_2$), 51.29 (NH*C*H), 52.41 (O*C*H$_3$), 53.10 (O*C*H$_3$), 66.83 (*C*H$_2$Ph), 80.30 (*C*HO), 127.82, 127.94, 128.26 (aromatic *C*H's), 135.91 (quaternary aromatic C), 151.17 (*C*=N), 155.83 (O*C*ON), 160.65 (*C*OC=N), 171.78 (CH*C*O$_2$Me); exact mass 364.1279 (364.1271 calcd for $C_{17}H_{20}N_2O_7$); Anal. Calcd for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 55.83; H, 5.45; N, 7.60.

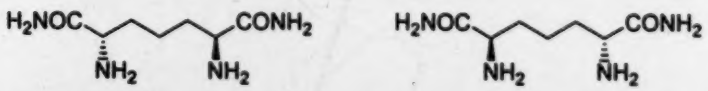


2,6-Bis(*N*-(benzyloxycarbonyl)amino)pimelic Acids (131a, b, c). The procedure of Arnold was adapted.[141] A solution of 2,6-diaminopimelic acid (19.2 g, 100 mmol) in 2 M NaOH (250 mL) was cooled to 0 °C and stirred vigorously while benzyl chloroformate (39 mL, 270 mmol) was added dropwise over 30 min. Vigorous stirring was continued for 3 h at ambient temperature, and the mixture was extracted with EtOAc (2 x 200 mL). The aqueous layer was cooled on ice/H_2O and acidified to pH 1 with 1 M

HCl. The mixture was extracted with EtOAc (3 x 200 mL), and the organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give a mixture of all stereomers **131a, b, c** (40.3 g, 88%).

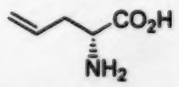
A portion of the above crude **131a, b, c** (25.0 g)was recrystalized thrice from boiling EtOAc (cooling to 0 °C) to provide white crystals of **131b** (LL-isomer) and **131c** (LL-isomer) which were filtered, washed with a little chilled (-20 °C) EtOAc and dried *in vacuo* (8.02 g, 64%).

All of the EtOAc filtrates and washings were pooled and evaporated *in vacuo* to give a gummy residue which was recrystallized from hot $CHCl_3$ (cooling to 4 °C, 3 weeks). The white solid obtained was further purified by recrystalization from hot CH_3CN (cooling to 4 °C, 2 weeks) to generate *meso*-isomer **131a** (6.2 g, 50%): IR (CH_2Cl_2 cast) 3500-2300 (br), 1717, 1529, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.1-2.1 (m, 6 H, $CH_2CH_2CH_2$), 4.35 (m, 2 H, 2 x CH), 5.09 (s, 4 H, 2 x CH_2Ph), 5.80 (br s, 2 H, 2 x NH), 7.26-7.32 (m, 10 H, 2 x Ph); FAB MS *m/z* (Cleland) 459 ($[M+1]^+$).

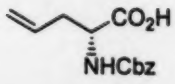


(2*S*, 6*S*)-2,6-Diaminopimelic Acid Diamide (132b) and (2*R*, 6*R*)-2,6-Diaminopimelic Acid Diamide (132c). The procedure of Arnold was adapted.[141] To a stirred solution of DD- and LL- *N*-Cbz-DAP **131b, c** (6.80 g, 14.7 mmol) and triethylamine (4.14 mL, 29.3 mmol) in 1,4-dioxane (90 mL) at 0 °C, was added isovaleryl chloride (3.6 mL, 29.3 mmol) dropwise. The mixture was stirred for 1 h at 10 °C and concentrated aqueous ammonia (3.9 mL, 54 mmol) was added dropwise. A precipitate formed and the mixture was allowed to stand 4 h at 4 °C. 1 M NH_4OH (5 mL) was

added, the slurry was filtered, and the solid was washed with 1 M NH_4OH and H_2O. This material was dried and suspended in glacial acetic acid (80 mL) with 10% Pd/C (120 mg) under 1 atm of H_2 for 12 h. The catalyst was removed by passing through a bed of Celite and the filtrate was concentrated *in vacuo*. The syrupy residue was dissolved in H_2O (15 mL), and evaporated *in vacuo* to remove remaining HOAc to give a mixture of **132b** and **132c** (2.1 g, 46%): IR (KBr) 3500-2000 (br), 1692, 1620, 1560, 1406 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.25-2.25 (m, 6 H, $CH_2CH_2CH_2$), 4.06 (m, 2 H, 2 x CH); FAB MS *m/z* (Cleland) 189 ($[M+1]^+$).



D-Allylglycine (138). The procedure of Black and Wright was adpated.[164] To **44** (7.8 g, 49.6 mmol) was added 2 M HCl solution (100 mL) and this solution was brought to reflux for 3 h. The solvent was removed by evaporation *in vacuo* and the resulting white crystalline **138** (3.3 g, 58%) was recrystallized from H_2O/EtOH: $[\alpha]_D$ = +36.52 (*c* 4.0 H_2O) (lit[164] $[\alpha]_D$ = +37.1 ° (*c* 4.0 H_2O)) ; IR (nujol) 3171, 1723, 1587 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 2.61-2.79 (m, 2 H, CH_2CHNH), 3.84 (dd, 1 H, *J* = 7.2, 5.5 Hz, CHNH), 5.22-5.36 (m, 2 H, CH_2=CH), 5.70-5.86 (m, 1 H, CH_2=CH); ^{13}C NMR (D_2O, 75.5 Hz) δ 36.47 (CH_2CHNH), 56.15 (CHNH), 120.57 (CH_2=CH), 131.41 (CH_2=CH), 174.29 (COOH); exact mass 115.0635 (115.0633 calcd for $C_5H_9NO_2$); Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.90; H, 7.99; N, 11.95.



***N*-(Benzyloxycarbonyl)-D-allylglycine (139).** The procedure to make **5** was employed to convert **138** (2.5 g, 21.7 mmol) to **139** in 87% yield: $[\alpha]_D$ = -15.6 ° (*c* 1.01 $CHCl_3$) (lit[52] $[\alpha]_D$ = -13.0 (*c* 5.0 $CHCl_3$)); IR ($CHCl_3$ cast) 3320, 1720, 1521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.52-2.70 (m, 2 H, C*H*$_2$CHNH), 4.55 (ddd, 1 H, *J* = 4.1, 6.6, 8.0 Hz, C*H*NH), 5.22 (d, 1 H, *J* = 8.0 Hz, N*H*), 5.68-5.78 (m, 4 H, C*H*$_2$=CH and C*H*$_2$Ph), 5.68-5.78 (m, 1 H, CH$_2$=C*H*), 7.31-7.47 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 36.39 (*C*H$_2$CHNH), 53.19 (*C*HNH), 67.29 (*C*H$_2$Ph), 119.70 (*C*H$_2$=CH), 128.17, 128.29, 128.58 (aromatic *C*H's), 131.86 (CH$_2$=*C*H), 136.08 (quaternary aromatic C), 156.13 (NH*C*O$_2$), 176.32 (*C*OOH); exact mass 249.10026 (249.1001 calcd for $C_{13}H_{15}NO_4$); Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.87; H, 6.31; N, 5.49.

Determination of Protein Concentrations. Protein concentrations were determined with the BioRad protein assay dye reagent (Commassie Blue, Bradford method) using a modification of the recommended assay procedure.[176] Standard curves (mg/mL) were prepared using an aqueous solution of bovine serum albumin (0.40 mg/mL), assaying 3 x 7 samples: 5, 10, 15, 20, 25, 30, 40 μL (2, 4, 6, 8, 10, 12, 16 μg). The BioRad protein assay dye (200 μL, undiluted) was added to each sample in a cuvette, mixed with H_2O to make 1 mL. After being incubated for 15 min at room temperature, the samples were assayed at 596 nm against a reagent blank (200 μL of dye, 800 μL of H_2O). The standard curves were drawn by concentrations versus the averages of absorbances at 596 nm of three samples at each concentrations.

Activity Assay for DAP Dehydrogenase. The procedure of Misono et al. was followed.[125] *meso*-DAP dehydrogenase activity was assayed at 25 °C in buffer B (0.2 M glycine, 0.2 M KCl, at pH 10.5) containing 1.0 mM of NADP, and 10 mM of *meso*-DAP at pH 10.5 in 1 mL cuvette using Hewlett Packard (HP) 8452A diode array spectrophotometer. The scan conditions are: 336-344 scan wavelength with 400-420 nm reference wavelength; runtime of 120s ($t_o = 0$); cycle 1.0s, integral 0.5s; temperature 25 °C. A unit of activity is 1 μmol/min NADPH formed under these conditions. 5 μL (24 μU) of DAP dehydrogenase solution was used. For kinetic studies of DAP dehydrogenase on *meso*-DAP, DAP dehydrogenase (500 μU) was assayed at 25 °C in buffer B containing 1.0 mM of NADP at pH 10.5, using 8 samples of *meso*-DAP concentrations from 0.12 and 6 mM.

Isolation of DAP Dehydrogenase. The procedures of Misono et al. were followed[126] and were performed at 4 °C unless otherwise stated.

1. Crude Extract. The DAP dehydrogenase was isolated from *Bacillus sphaericus* IFO 3525 (cultured in Department of Microbiology by Professor Michael Pickard and Mr. Richard Mar). Cells were filtered, washed with 10 mM of potassium phosphate buffer containing 1 mM of DTT at pH 7.4 (buffer A), and centrifuged (Sorvall RC5B centrifuge, GSA rotor) to give a total wet weight of 154 g. The pellets were resuspended in buffer A by pumping through a syringe and the suspended solution was centrifuged (30 min at 10,000 rpm). The supernatant was discarded and the pellets were re-suspended again. The cells in the resultant solution were broken by sonication in a continuous flow

sonicator (3 x 1 min passages) chilled to 0 °C. Cell debris was removed by centrifugation (30 min for 10,000 rpm) and the supernatant was collected (600 mL).

2. Ammonium Sulphate Cuts. The extracts were dialyzed against buffer A (8 L) using Spectrapor™ membrane tubing size 4. (6.4 mL/cm) for 14 h, then 5 h. The supernatant was transferred to a beaker and the tubing was washed with buffer A (100 mL) which was combined with supernatant to make up 695 mL. To this solution, 168.8 g of $(NH_4)_2SO_4$ (40% saturation) was added slowly with stirring at 4 °C within 50 min and it was allowed to stand for another 10 min. A mixture of protease inhibitors (1.0 mM PMSF, 1 μM Pepstain A, 1 mM EDTA) was also added. The precipitate was removed by centrifugation (30 min at 10,000 rpm) and to the combined supernatant in a beaker was added a second portion of 142 g of $(NH_4)_2SO_4$ (80% saturation) within 40 min. The precipitate was removed by centrifugation again and this 40-80% cut was redissolved in 100 mL of buffer A which was dialyzed against the same buffer (4 L) for 14 h, then 5 h and 14 h.

3. DEAE-52 Cellulose Chromatography. DEAE-52 cellulose anion exchange column was packed with 300 mL of buffer A. The column was first washed with 0.2 M of NaCl in buffer A (350 mL) at 1 mL/min and then equilibrated with buffer A (no NaCl) for 12 h (0.9 mL/min, 800 mL). The above dialyzed enzyme solution was loaded on the column and the column was eluted with a NaCl gradient of 0 - 0.25 M in buffer A with protein detection at 280 nm, collecting 10 mL fractions. Fractions were assayed for protein concentrations and activity. Those fractions with high activity were combined and concentrated by $(NH_4)_2SO_4$/dialysis to a final volume of 100 mL.

4. Hydroxyapatite Column. The hydroxyapatite column was washed with buffer A (350 mL) at 1 mL/min and then 1 mM potassium phosphate buffer containing 1 mM DTT (1500 mL) at 1 mL/min. The enzyme solution after DEAE-52 column was applied on the hydroxyapatite column, eluted with 1 mM of potassium phosphate buffer (700 mL), 20 mM buffer (600 mL), 30 mM buffer (600 mL) and finally 50 mM buffer (400 mL) at 1 mL/min. All active fractions were combined and concentrated to final volume of 50 mL. Measurement of protein concentration and activity levels indicated the activity was 4.84 units/mL and the specific activity was 2.8 units/mg.

Test of Compounds as Substrates for DAP Dehydrogenase. Substrate tests were done in buffer C (0.1 M Tris-HCl, 1 mM EDTA at pH 7.8) containing 0.25 mM NADP, DAP dehydrogenase (480 μU) and 0.33 mM potential substrates at pH 7.8, using runtime of 600s by monitoring 336-344 scan wavelength with 400-420 nm as reference wavelength. The blank contained: 0.25 mM NADP, 480 μU DAP dehydrogenase.

Test of Compounds as Inhibitors for DAP Dehydrogenase (IC_{50}). Compounds were tested at pH 7.8 and pH 10.5 separately. At pH 7.8, compounds were assayed at 25 °C in buffer C containing 0.25 mM NADP, DAP dehydrogenase (480 μU) and 0.33 mM *meso*-DAP, using potential inhibitor concentrations from 10 μM to 10 mM. At pH 10.5, tests were in buffer B with 1.0 mM of NADP, 10 mM of *meso*-DAP and concentrations of potential inhibitors were from 10 μM to 10 mM.

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